PROSPECTIVE OBSERVATIONAL STUDY TO DETERMINE THE FACTORS ASSOCIATED WITH OUTCOME OF SCRUB TYPHUS

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF M.D. GENERAL MEDICINE BRANCH I EXAMINATION OF THE TAMIL NADU DR M.G.R. UNIVERSITY, CHENNAI TO BE HELD IN 2016.

CERTIFICATE

This is to certify that the dissertation "**Prospective observational study to determine the factors associated with outcome of Scrub Typhus**" is a bonafide work of Dr Rohit Barnabas carried out under our guidance towards the M.D. Branch I (General Medicine) Examination of the Tamil Nadu Dr M.G.R. University, Chennai to be held in 2016

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This is to certify that the dissertation titled "**Prospective observational study to determine the factors associated with outcome of Scrub Typhus**" which is submitted by me in partial fulfillment towards M.D. Branch I (General Medicine) Examination of the Tamil Nadu Dr M.G.R. University, Chennai to be held in 2016 comprises only my original work and due acknowledgement has been made in text to all material used.

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I am forever grateful to all the patients who agreed to be part of this study.

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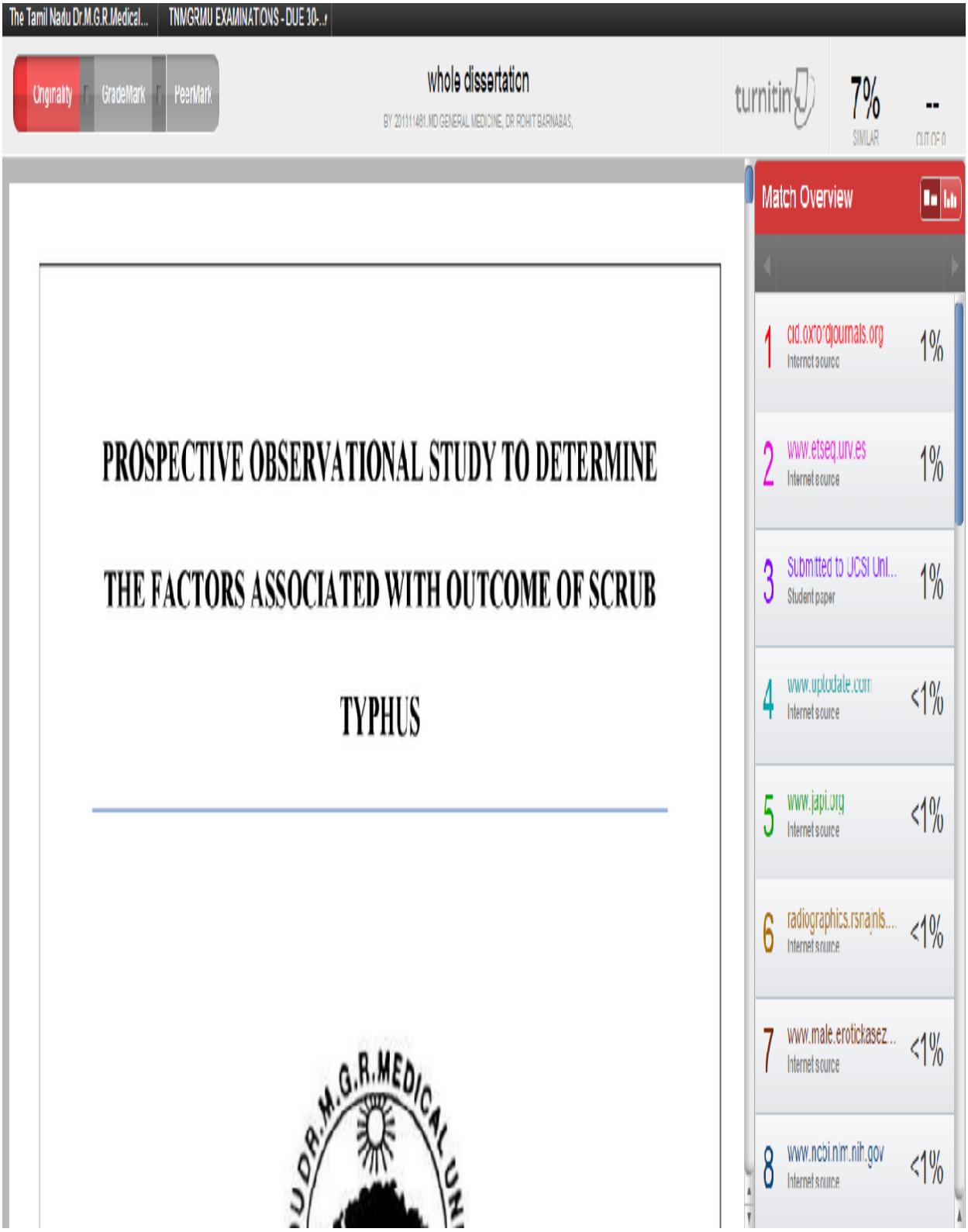
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Prospective observational study to determine the factors associated with outcome of scrub typhus.
Dr. Rohit Barnabas, PG Registrar, Dr. K.P.P Abhilash, Dr. George. M. V, Dr. Shubanker, Dr. Sudha, Dr. Ramya, Dr. Thomas. I, Medicine, Dr. John Jude, Microbiology.

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Dear Dr. Rohit Barnabas,

I enclose the following documents:-

1. Institutional Review Board approval
2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

CC: Dr. K.P.P Abhilash, Medicine, CMC.

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Ref: IRB Min. No. 8488 [OBSERVE] dated 09.10.2013

Dear Dr. Rohit Barnabas ,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project entitled "Prospective observational study to determine the factors associated with outcome of scrub typhus." on October 9, 2013.

The Committees reviewed the following documents:

1. IRB application form
2. Curriculum Vitae' of Drs. Rohit Barnabas, K.P.P Abhilash, George. M. V, Shubanker, Sudha, Ramya, Thomas. I, John Jude.
3. Informed Consent form (English, Hindi & Tamil)
4. Data abstraction sheet
5. Patient Information Sheet (English, Hindi, Telugu & Tamil)
6. No of documents 1-5

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on October 9, 2013 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

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Dr. Vathsala Sadan	M.Sc, PhD	Professor, Community Health Nursing, CMCH.	Internal, Nurse
Rev. Joseph Devaraj	B. Sc, BD	Chaplaincy Department, CMCH.	Internal, Social Scientist

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We approve the project to be conducted as presented.

The Institutional Ethics Committee expects to be informed about the progress of the project, any **adverse events** occurring in the course of the project, any **amendments in the protocol and the patient information / informed consent**. On completion of the study you are expected to submit a copy of the **final report**. Respective forms can be downloaded from the following link: http://172.16.11.136/Research/IRB_Polices.html in the CMC Intranet and in the CMC website link address: http://www.cmch-vellore.edu/static/research/Index.html.

Fluid Grant Allocation:

A sum of 10,000 INR (Rupees Ten Thousand only) will be granted for 2 years.

Yours sincerely

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

CC: Dr. K.P.P Abhilash, Medicine, CMC.

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INTRODUCTION

Scrub typhus is a zoonotic bacterial infection, which is a chigger borne rickettsiosis. It is an important cause of acute febrile illness in South East Asia and South Western Pacific regions(1).It is a reemerging bacterial infection. The causative bacterium, Orientia tsutsugamushi is transmitted to humans and rodent hosts by the bite of the larval stage of trombiculid mites (Vector) *Leptotrombidium deliensis* called chiggers. The Vector is seen in a wide variety of ecological regions from the mountainous regions of northern India to the tropical climates of the Malay Peninsula, and Indonesia. The clinical features include acute presentation of fever, myalgia, headache, Mutiple organ dysfunction with an eschar at the site of inoculation in variable proportion of patients(2).It can range in severity from a mild disease, self remitting disease to a fatal illness with 30-50% mortality(3) if untreated in earlier studies and 7.8-9% noted in recent data(2,4). World health organization (WHO) has declared it a re-emerging disease and has called for increased surveillance. They can have an elevated hepatic enzymes, creatinine, and bilirubin. They may have thrombocytopenia, leucopenia or leukocytosis. It can be confirmed using serology using the indirect fluorescent antibody assay, biopsy of the eschar, culture and polymerase chain reaction on the tissue, eschar and serum(5).

Scrub typhus is diagnosed based on clinical evidence and appropriate laboratory features. There is no laboratory test that is reliable in the early stage of the disease.

Scrub typhus lasts for 14 to 21 days without therapy. Death may occur in the late second week due to the complications(6). Patients treated with appropriate therapy show fever defervescence within 48 hours after the initiation of the therapy, this

response is considered characteristic for diagnosis of scrub typhus. The various groups of antimicrobials used are tetracycline (Doxycycline), macrolides (Azithromycin), Chloramphenicol. Failure of defervescence within 48 hours is suggestive of an alternative diagnosis for the acute febrile illness. However it has been noted in the recent studies to have a delay in the defervescence duration with appropriate therapy. In a study done in 2004 showed that 20 of the 93 patients had a delay in defervescence in the fever duration beyond 48 hours(7). A retrospective study from china in 2008 showed that 18 out of 88 patients had a delay in fever defervescence beyond 48 hours(8). The above said studies assessed the role of therapy in mild disease with no studies in patients with severe disease. The proposed studies aims to study the various antibiotics used, incidence of delayed defervescence and the factors affecting the same.

AIM

To study the factors associated with outcome and comparison of antibiotic regimens in the treatment of scrub typhus.

OBJECTIVES

1. To find the incidence of delayed defervescence in all scrub typhus patients.
2. To determine and compare the different antibiotics used for the treatment of scrub typhus.
3. To determine the factors associated with delayed fever defervescence.
4. To determine the pattern of resolution of organ involvement in patients with severe scrub typhus with multi-organ dysfunction.

LITERATURE REVIEW

Scrub typhus is a mite borne infectious disease caused by *Orientia tsutsugamushi*. The disease was described by the Chinese in the third century but the first descriptions were seen in the western literature in the nineteenth century. Knowledge about scrub typhus increased in the Second World War era when the soldiers had a common occurrence in the pacific fight mainly in the Vietnam and the indo china border.

HISTORY

Scrub typhus has been described by Chinese investigators since third century A.D. It was first reported by Hashimoto in 1810 in Japan as a life threatening disease occurring in the northern island of Honshu along the river banks(9). The Japanese associated the disease with bite of a jungle mite named tsutsugamushi which is for dangerous bug in Japanese. Heterophile antibody for scrub typhus was first demonstrated by Fletcher and Lesslar in 1926. The causative organism was first isolated in 1931 by Ogata and was named by which it is known today, Orientia tsutsugamushi in1996(10).The disease was associated with altered sensorium(typos for fever with stupor in Greek) and was associated with scrub lands hence the name scrub typhus.

Scrub typhus occurred in periodic outbreaks in the early 1900s, it was reclassified as typhus like illness fever in 1917. The disease had a very high case fatality rate approaching 50% in untreated cases causing thousands of deaths in the World War II era. The local name for the illness in Japan continues to be tsutsugamushi, with other

names being akamushi (red mite) fever and kedani (hairy mite) fever also noted. The resurgence of scrub typhus is seen in various parts of India and neighbouring countries.

EPIDEMIOLOGY

Organism:

Scrub typhus is caused by O. Tsutsugamushi. It is a gram negative obligate intracellular bacterium.

It was earlier classified under genus Rickettsia based on the following characteristics shared with the other members of the genus 1) basic morphology, 2) exhibited obligatory intracellular parasitism, 3) required an arthropod for human transmission, 4) ability to survive in both vertebrate and invertebrate host. However Tamura et al proposed it be reclassified under a new genus, Orientia based on several differentiating factors namely 1)16s RNA sequencing showed it to be different from other rickettsial, 2)electron microscopy showed a thicker outer leaflet of the cell wall with ill formed flagella and endospores, 3) lack of peptidoglycan and lipopolysaccharides, such as muramic acid, glucosamine, hydroxyl fatty acid, 2 keto 3 deoxytonic acid, making the cell structure fragile and penicillin resistant, 4) gel electrophoresis showed abundance of major cell surface 56 KDa protein and 60 KDa protein, 5)growth in cell lines demonstrated phenomenon of budding similar to enveloped virus with absence of electron lucent halo zones around growing cells and

6) Heterophile antibodies to OXK antigens on the weil felix test. In 1996, genus Orientia was classified consisting of a single species Orientia tsutsugamushi(11).

ENDEMICITY

It is seen in the terrain areas of the 'tsutsugamushi' triangle which is a geographical region comprising South East Asia and Southwest Pacific (1). Scrub typhus was known as early as the 3rd century in China; however the magnitude of the disease was evident in the 2nd world war as many soldiers were victims of the chigger mite borne disease.

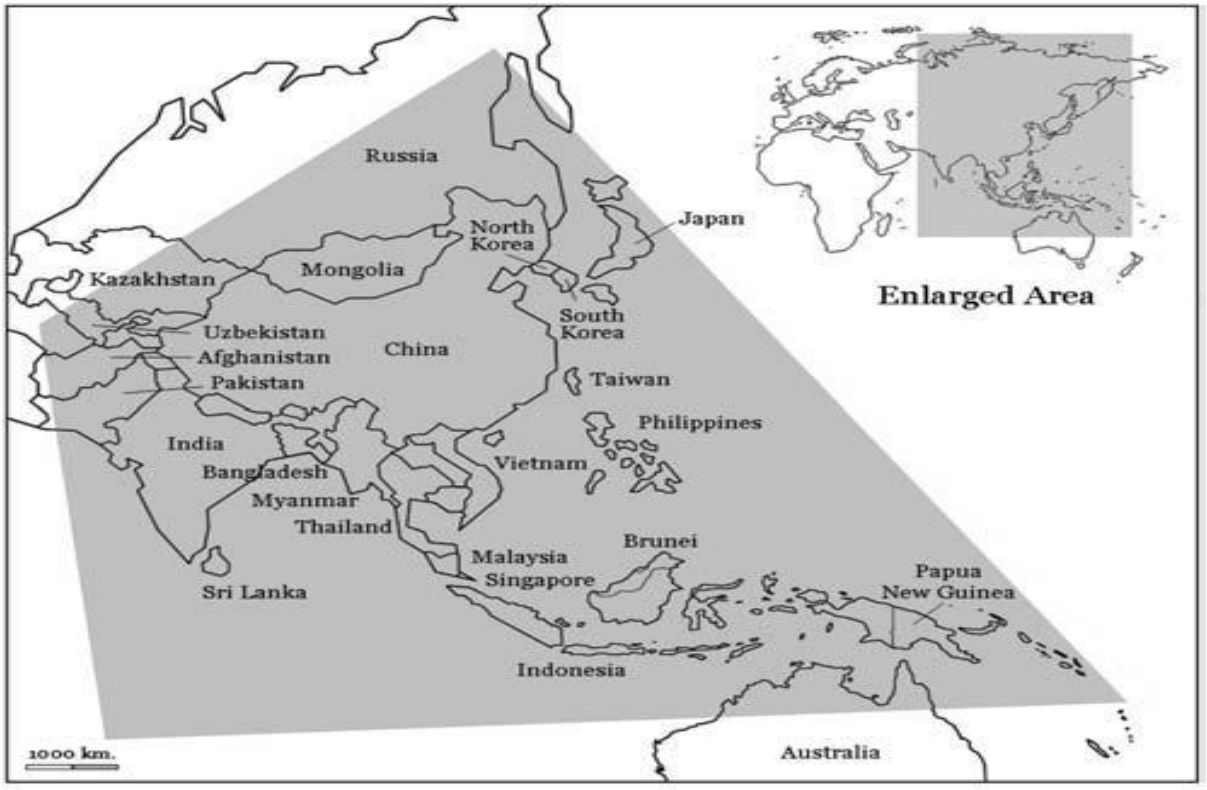


Figure 1: "The Tsutsugamushi Triangle"

Though whole of India is reported to be endemic for the disease, the reporting of the disease has seen a recent surge since 2004 from different parts of the country. Scrub Typhus is mainly noted in areas which are scrubby and are forested, which are the usual reservoirs of rodents and mites (seen in figure below)(12).

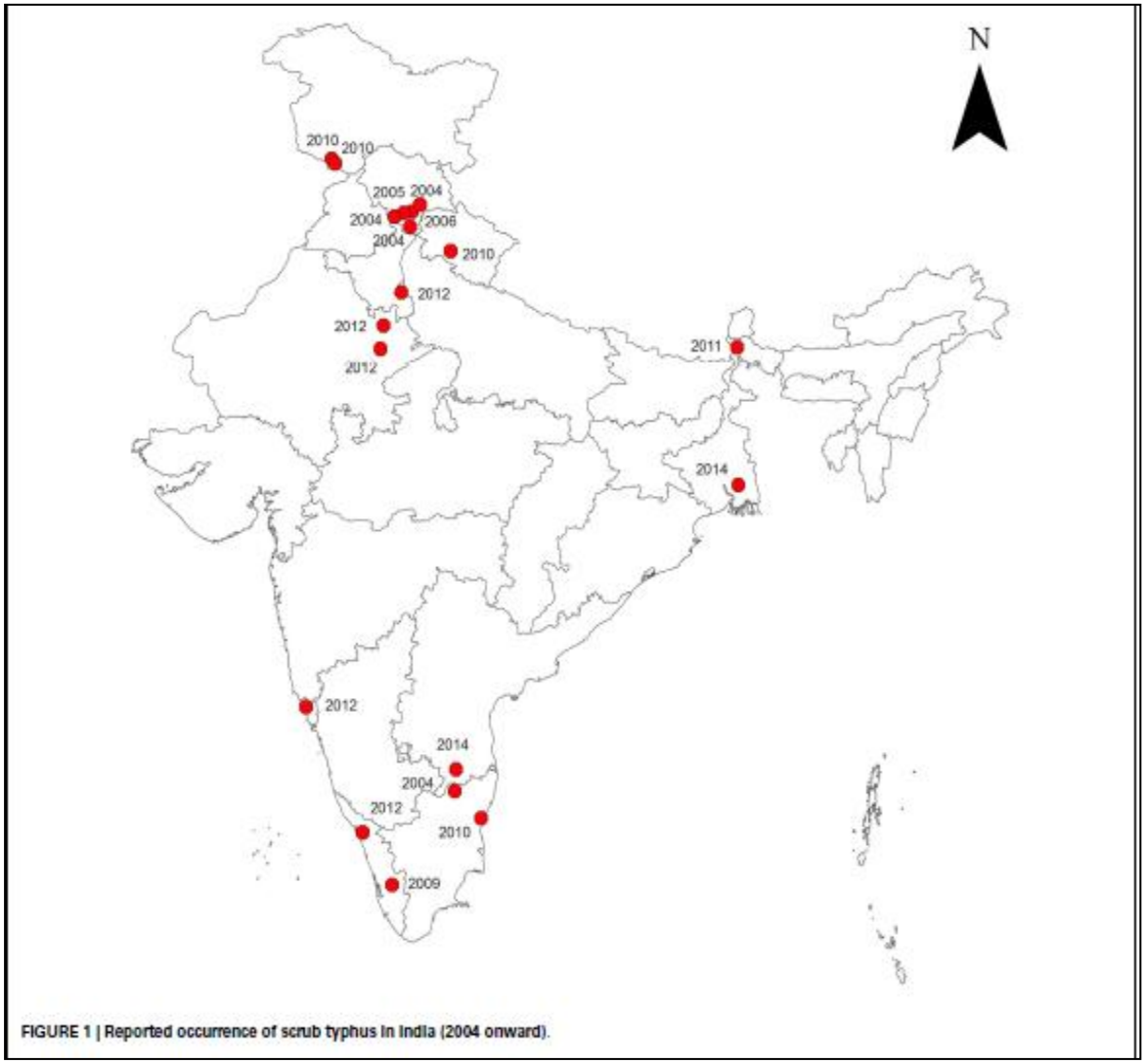


Figure 2: Endemicity of Scrub Typhus in India

MICROBIOLOGY

The organism is a gram negative, Giemsa positive coccobacillus that is antigenically different from the Typhus group of Rickettsia. It measures 0.5-0.8µm X 1.2µm in size. It is surrounded by a cell wall around the cell membrane. The cell wall is made up of a major surface protein which is made up of a 56kDa substance comprising 10-15% proteins. The cell wall also has a 60kDa protein which is related to GroEL family of protein which is mainly seen in the eukaryotic bacteria. It does not have a slimy layer, flagella formation or sporulation. The cell wall does not have peptidoglycan or lipopolysaccharides such as muramic acid, glucosaminoglycans, and deoxytonic acid and hydroxyl fatty acid.

There are more than 20 different antigenic strains of O. Tsutsugamushi reported. The clinical virulence is noted to be different with strain variation. Direct antigenic characterisation can be done using 2 methods.

1. Black plate collection method: flat, square, black plastic tiles are placed on the ground; unengorged chiggers that attach to the plates are removed for rickettsial isolation.

2. Chiggers infected are removed from the ears of the field trapped rodents and are processed for Orientia strains.

There are various methods of serological characterisation which include complement fixation, toxin and serum neutralization, indirect fluorescent antibody assay and direct antibody fluorescent assay. The other recent techniques used which use other than

56kDa cell surface antigen genes are gel electrophoresis, restriction fragment-length polymorphism (RFLP), Polymerase chain reaction(1).

Methods of Characterisation of Antigenic strain:

Complement fixation (CF): CF is one of the earliest methods for clinical diagnosis of scrub typhus. It was used by shishido et al to confirm the identity of 3 original strains Karp, Gilliam and Kato strains. The principle of the test is that the amount of complement fixed is proportional to the mass of the antigen- antibody complex of the Orientia antigen. This test showed specificity with absence of cross-reaction with non scrub typhus group antigens.

Cross-neutralization and cross-immunization: This method is very cumbersome. In cross neutralization assay serum from specific antibody is taken from hyperimmunised animals are incubated with Orientia organisms. The serum is injected into susceptible mice, infectivity is observed by the reducing ability of specific neutralizing antibody. It can also be compared by the monitoring cytopathic effect.

The cross- vaccination or cross-immunization mice and guinea pigs are immunised with live or killed Orientia organism by a non lethal route such as subcutaneously. The strain variation can be characterised by looking at the Virulence of the inoculated animals. The strain variation can also be made out by using a toxin neutralization assay using with egg yolk sac viability.

Immunofluorescence and immunoperoxidase assays: The initial sequencing of gene was done using direct fluorescent assay(DFA) and indirect fluorescent

assay(IFA).The strains used in the assays are anti-Karp, -Kato, -Gilliam, -TA678, -TA686, -TA716, -TA763, and -TH1817 serum.

Monoclonal antibody typing: Antibodies are specifically derived from reactive with the prototype strains.

SDS- PAGE immunoblot assay: This technique uses electrophoresis from multiple strains of Rickettsia isolated from many different strains. The 56kDa protein is the most studied antigen for the electrophoresis, the other antigens used are 110kDa, 70kDa, 60kDa, 56kDa, 47kDa, and 22-25kDa.The 56kDa antigen is located on the cell surface and is unique and has cross reactive epitopes, this is a specific protein which consists of 520 amino acids but can vary between 500-540 amino acids in different strains. The characteristic type specificity 16SrRNA was used to place scrub typhus in the new genus Orientia(1).

The original prototype strains isolated were Karp and Gilliam (1943) and Kato (1955) in New Guinea, Assam-Burma border and Japan respectively. Many strains have been isolated from the endemic areas which include Shimokoshi, Kawasaki, and Kuroki from Japan and Boryong from Korea. The strain variation depends on 2 major proteins namely 110kDa and 56kDa major surface membrane protein.

56kDa protein is a type specific antigen. Gene sequencing of this antigen has shown great genetic diversity between strains. The locus contains 4 hyper variable regions (VDI-VDIV). Altered base pairs in this region give rise to specific protein products which are strain specific and immunologically different. Phyllogenetic analyses have provided information regarding genetic diversity of various strains and their

geographical location. Gene coding for Karp strain was done by Stover in 1952(13).According to the genotypic study system in Japan O tsutsugamushi was classified into Gilliam, JG(Japanese Gilliam), Karp, JP-1(Japanese Karp 1), JP-2(Japanese Karp type 2), Kato, Kawasaki, Kuroki, Shimokoshi, and others. Kelly et al in their review of O. Tsutsugamushi strain distribution in the Asia – pacific region, have reported that each geographic focus has a predominant strain type. Overall karp, Karp-like and Gilliam- like strains seems to be most prevalent(1).

In India, Gilliam type strain was isolated by Bengston et al in Assam-Burma region in 1943(14).The sera tested from outbreak in 1944 in Indo-Burma border showed that infection with multiple strains noted were Gilliam(36%), followed by Karp(7%) and Seerangayee (7%). In 2004 a study from Himachal Pradesh showed the strains identified on PCR were Karp and JP1 and Satitama and JG type(15).In a recent study done in 2007 where 66 samples were tested by RT PCR(real time PCR) showed the strain to be close to Kuroki type(16).In a study done in our institution in 2013 on 26 samples showed 17(65%) to be of Kato type and 8(31%) to be of karp like group(2).

Nayakayama et al have completed the sequencing of two strains namely Boryong and Ikeda; it has a single circular chromosome made up of 2 million base pairs with G+C pairs forming almost 30% of the base pairs. The genome had about 4197 identical base pairs. Major proportion of the high copy number tandem repeats coded for mobile genetic elements including conjugative transfer genes, transposons and phage genes .Of these 359 tra genes coding for Type IV secreting system (TFSS), which is a conjugative system for transfer of DNA from one cell to another, were identified, Presence of such a system in an intracellular bacteria, like O. tsutsugamushi, is

unusual and may facilitate both vertical inheritance(in primary host) and horizontal transfer in secondary ones (man, rodents)(17)(18).

Table 1:Epidemiological prevalence studies showing geographic distribution of predominant strains, Adapted from Kelly et al(1).

Geographic location, isolate sources (no. of isolates)	Method(s)	Predominant strain(s)[a]
Queensland and Northern Territory, Australia		
Human (27), rodent (21), and mite (4)	DFA	TA716, in North Queensland (94%)
Pescadores, Taiwan		
Human (17), rodent (17), and mite (15)	DFA	TA716 (100%) and Karp (47%)
Fujian Province, China		
Human, rodent, and chigger (126 total)	CF, DFA, and IFA	Karp (23%), Gilliam (10%), and TA716 (1%); Karp mix (72%)
Shandong Province, China		
Human, rodent, and mite (23 total)	IFA, MAb, and RFLP	Gilliam (91%) and Karp (9%)
Human, rodent, and mite (21 total)	56-kDa Seq	Kawasaki (95%) and Kato (5%)
Korea		
Human (113)	IFA and MAb	Boryong, primarily in Chungnam Province (78%)
Akita, Niigata, Kanagawa, Shizuoka, Oita, and Hokkaido prefectures, Japan		
Human (3), mite (89), and rodent (70)	CF	Karp (53%), Gilliam (31%), Kato (11%), and mixed antigenicity (2%)
Niigata, Kyoto, Tokushima, Akita, Miyazaki, Gifu, and Shizuoka prefectures, Japan		
Human (15), rodent (3), and mite (2)	MAb, RFLP, and 56-kDa Seq	Karp (25%), Gilliam (20%), and Kawasaki (20%)
Shizuoka Prefecture, Japan		
Human (24), rodent (30), and mite larva (5)	MAb and IFA	Karp (60%), Kawasaki (34%), and Kuroki (7%)
Niigata, Akita, Kanawagawa, Toyama, Saitama, and Fukushima prefectures, Japan		
Human, rodent, and mite (22 total)	MAb, IFA, and IP	Karp (64%) and Gilliam (36%)
Primorski Krai, Russia		
Human (1), rodent (1), and mite (1)	IFA, CF, and CN	Gilliam (100%)
Philippines		
Febrile human serum (23)	CF	Karp (78%), Gilliam (83%), and Seerangayee (61%)
Rodent (42)	DFA	TA716 (86%), TA686 (81%), and Karp (67%)
Myanmar (Burma)		
Serum from patients with fever (14)	CF	Gilliam (36%) and Karp-Seerangayee mix (46%)
Malaysia		
Human (114)	DFA	TA763 (72%), TA716 (70%), and Karp (56%)
Mites direct[b] (168)	...	Karp (93%)
Tadzhikistan		
Rodent (4) and mite (4)	CF	Gilliam (100%)
Thailand		
Human, rodent, and mite (77)	CF	Karp alone (43%); Karp and mix of ≥2 others (67%)
Mites direct[b] (146)	DFA	Karp (51%)
Human (27)	56-kDa Seq	Karp-like (65%), Gilliam-like (22%), and TA716 (4%)

Vector and its life cycle:

The etiological agent of scrub typhus is Rickettsia, the reservoir for which is mainly the rat, field mouse, or the bandicoot. The organism is transmitted to man by the bite of larval form of the mite Trombiculia akamushi, T. deliensis, and T. hirsti. The larval mite is seen in grass, brush, and secondary jungle growth consisting of stunted trees namely scrubs. The mite acquires the organism by sucking on the infected vertebrate host(19). There are several species of Leptotrombidium, each with its characteristic geographic distribution. L. deliense appears to be the primary vector in most countries including India. There was a new species of vector for scrub typhus was noted in Darjeeling namely Schoengastiella lingual as the vector of scrub typhus.

Table 2: Putative vectors of human scrub typhus and their geographic foci(1).

Vector[a] (*Leptotrombidium* species)	Localities
L. deliense	Prevalent in Australia [3, 4], China [3, 5–8], India [3, 9], Malaysia [3, 10], New Guinea [3, 11], Pakistan [1, 3], Philippines [3, 10], and Thailand [3, 12, 13]; present in Sumatra (Indonesia) [3], Myanmar [3], and Pescadores islands (Taiwan) [3]
L. akamushi	Prevalent in Japan [1, 3, 10, 14–16] and Solomon Islands [3, 17, 18]
L. scutellare	Prevalent in Japan [1, 3, 10, 14–16]; present in China [6], Korea [19], Malaysia [3, 20], and Thailand [3, 12]
L. chiangraiensis	Present in Thailand [21, 22]
L. arenicola	Present in Indonesia [3] and Malaysia [1, 3, 23]
L. imphalum	Present in Thailand [3, 21]
L. pallidum	Prevalent in Japan [1, 3, 10, 14–16] and Korea [1, 3, 24, 25]; present in Primorski Krai (Russia) [1, 3, 16]
L. pavlovskyi	Prevalent in Primorski Krai (Russia) [3, 26, 27]
L. fletcheri	Present in Indonesia [1], Malaysia [1, 3, 28, 29], New Guinea [1], and Philippines [1, 3]
L. gaohuensis	Present in Zhejiang Province, China [5]

NOTE. Several other trombiculid mite species are not identified in the table, either because of relative rarity or because they are unproven human vectors [1]—for example, *L. palpale* (Japan, Korea, and Primorski Krai, Russia), *L. tosa* (Japan), *L. fuji* (Japan), *L. orientale* (Japan, Korea, and Primorski Krai, Russia), and *L. intermedium* (China).

[a] Primary chigger vectors of scrub typhus are found in the genus *Leptotrombidium* (*L. deliense* group). The mite life cycle is 2–3 months in warmer climates and ≥8 months in colder climates; only the parasitic, 6-legged larva or "chigger" stage parasitizes the vertebrate host [3].

LIFE CYCLE

Humans acquire the disease by the bite of an infected mite. The adult mite has four staged lifecycle namely:

1. Egg
2. Larva
3. Nymph
4. Adult.

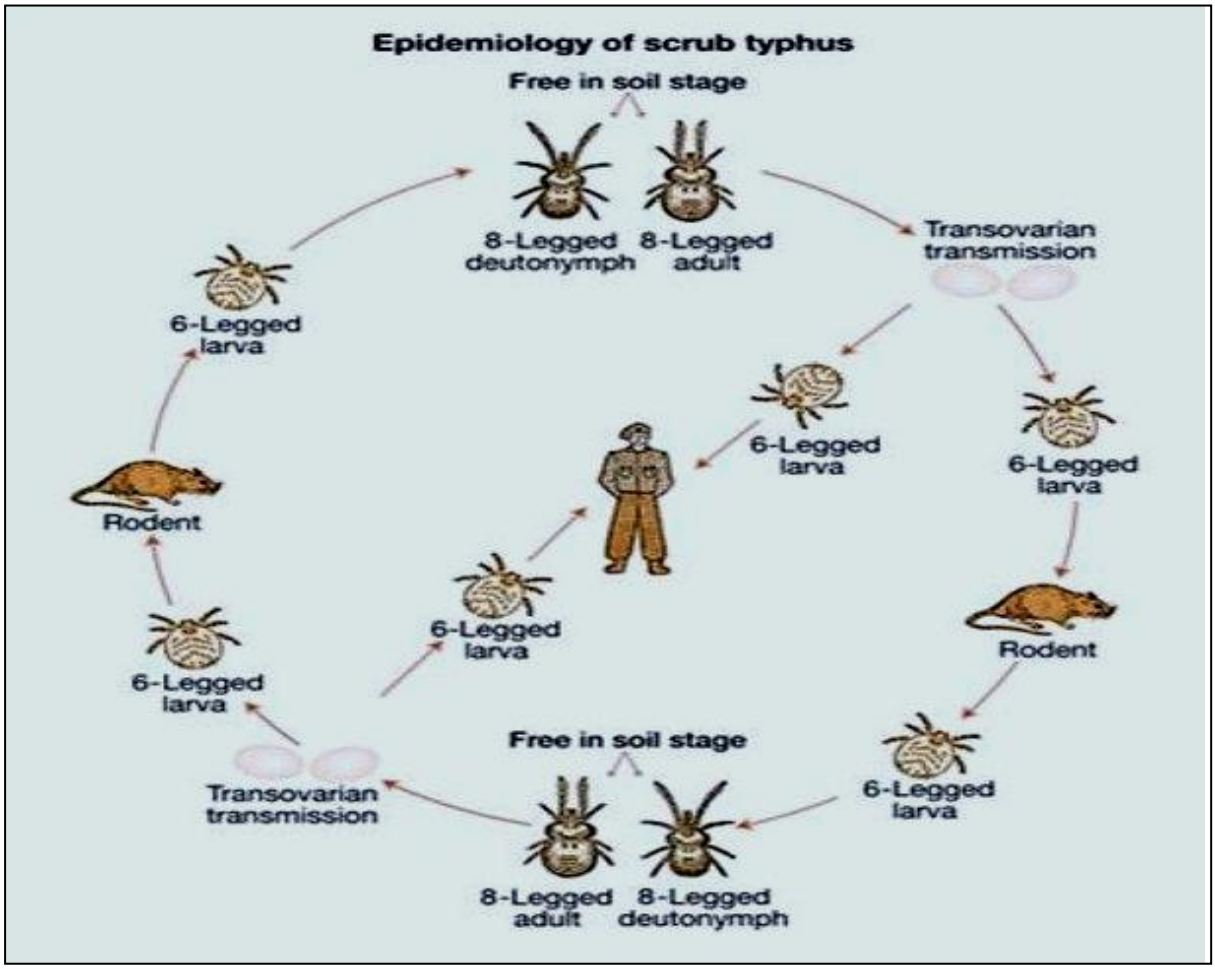
The egg deposited by the adult female develops into larval form called chiggers. They aggregate to form the mite islands /typhus islands which are mainly seen in the shrubs and the grasslands awaiting hosts such as rodents and small animals. These larval forms feed on the meal of the blood of the vertebrate host thereby acquiring the disease and transmitting the pathogen. Once engorged with blood the chigger falls off from the body and continues to form nymphal and then adult forms. The larval stage of the organism feeds only once in the life time (figure on the life cycle).

The larva also called as the chigger is the only stage that can transmit the disease to humans and other vertebrates, as the other stages do not feed on vertebrate animals. Chigger mites act as the primary reservoir for the organism. They are infected by feeding on the bodily fluid of the infected vertebral host. The infection is passed on to all the stages of the lifecycle by transovarial transmission, where the infection is passed on to from adults to other stages through the egg. The infection is also passed on from the egg to the larva is called transtadial transmission. Earlier studies thought

rodents as the natural reservoir, however recent studies have revealed that the mites act as the vector and also the reservoir(20).

Humans act as the dead end host with no human to human transmission. The disease is seen more commonly as there is encroachment of the scrub land by the agricultural and wartime activities making soldiers and farmers more susceptible for the acquiring of the disease(1).

Figure 3: Life cycle of Scrub Typhus



PATHOGENESIS:

O. tsutsugamushi survives in the wild in a cycle involving trombiculid mites and other vertebrates. Humans act as the accidental hosts for the organism. O tsutsugamushi is

different from rickettsia in the genetic makeup, cell wall structure. The organism acts as an obligate intracellular gram negative bacterium. The larval stage of the mites (chiggers) harbouring the bacterium after biting the exposed individuals multiplies at the site of inoculation causes local and systemic manifestations.

O. tsutsugamushi inoculated in the saliva of the chigger after the bite to the skin mainly enters the dendritic cells and macrophages in the dermis of the underlying eschar at the site of inoculation. Attachment and entry of the obligatory intracellular organism is by the clatrin-dependent endosomal pathway which involves the 56kDA surface protein, host fibronectin autosomal transporter(ScaC), integrin-a5b1 and syndecan-4 host cell receptors(21).

Immune response

The organism induces both humoral and cell mediated immunity. The immune response is complicated in view of great antigenic diversity. Homologous immunity is provided by the major surface antigen, 56 KDa protein molecules that contain strain specific epitope. The antibodies to 56kDa molecule increase uptake by the macrophages and neutrophils and cause clearance of the organism. The protective immunity is mediated by the TH1 response. The various cytokines which rise during the acute infection are macrophage colony stimulating factor (M-CSF), interferon gamma (IFN-V), and granulocyte colony stimulating factor (G-CSF), interleukin 1beta, IL-12p40, tumour necrosis factor alpha, IL-10, chemokines such as CXCL-9 and CXCL-10. Few patients also showed a rise in tumour necrosis factor (TNF – alpha), this tumour marker is also seen to increase in the convalescence period.

However the role of CD4 cells and CD8 cells, natural killer cells, NKT cells, dendritic cells. Macrophages and endothelial cells have not been evaluated in disease models(21).

There is also indirect evidence for cytotoxic lymphocyte activation, cytotoxic T cell and natural killer cell activation during acute infection. This plays a role in destroying the infected host cells.

The organism has developed mechanisms to evade the immune mechanisms. Cho et al showed that the organism down regulates the expression of the glycoprotein 96(gp 96) in the macrophages and endothelial cells. This molecule is expressed in the endoplasmic reticulum of the cells and plays a central role in the major histocompatibility complex class I antigen presentation; the suppression of this glycoprotein plays a major role in the neutralization of the host response(22).

It has three phases, first at the site of inoculation, second, systemic dissemination, endothelial dysfunction, and vasculitis lastly, antibody mediated phenomenon. Disseminated vasculitis is central to the pathogenesis of scrub typhus.

Inoculation- Eschar

The eschar is the initial site of inoculation, is a result inflammatory response to the bite of the chigger. The eschar produces in rapid order a papule, vesico-papule, excoriated papule, frank eschar, ulcer and finally a small scar. The lesion evolves over 3-4 weeks and can get prolonged in case of secondary infection such as in groin or axilla. The maximum diameter of the eschar is around 5mm and the surrounding erythematous area is also of the same diameter(19). The initial inflammation can

spread to the mid dermis with polymorphonuclear cells in the upper zones with increasing monocytes in the deeper zones. There is an acute thrombophlebitis and arteritis within the zone of inflammation surrounded by area of mononuclear infiltration within the venules.

Systemic dissemination

The endothelium forms the principle site of inoculation of the organism. Between 1916 and 1922 the giemsa stain demonstrated the rickettsia to be in the endothelial cells of the cutaneous biopsies and autopsies by Wolbach et al. Allen and spitz et al showed that the organism was noted in the eschar and also on some cases in the macrophages and spleen. The organisms are found in the endothelial cells of the myocardial capillaries and in cardiac myocytes. The cytoplasm of the capillary endothelial cells was consistently found to have the organisms. The rickettsia is known to exit from the host cell by budding from the plasma membrane(23). There is also evidence to show that the organism was seen within the mononuclear cells of the acute phase of the scrub typhus illness. There are also reports to show that the organism is seen in the dendritic cell/macrophages. The organism after inoculation into the skin spreads to the regional lymph node which is followed by systemic dissemination. The organism thus may use the haematogenous and also indirectly by the lymphatic system for systemic dissemination(24)(25).

Thus the organism mediates intracellular events leading to the generation of the reactive radical in the host cells, cytokines and increases cytotoxic immune cells

leading to the pathophysiological effects such as increased vascular permeability leading to edema, vasculitis(21).

The severity of the illness depends on both host and pathogen related factors. The bacterium multiplies in the principal target sites which is the endothelial cells. It affects the endothelial cells of the heart, lung, brain, liver, kidney, pancreas, skin. It also affects the macrophages of liver and spleen. The organism gains entry into the body through the lymphatic cells. It has also been demonstrated that there is a possibility of blood borne infection as the organism was found in the mononuclear cells(22).

Persistence of infection

The persistence of the organism was described in infected mice, where the organism was found in the body for up to 610 days after inoculation(26)(27). Smadel et al showed that the organism can be isolated from lymph node after 2 years after the infection of an asymptomatic person(28). Chung et al showed that the blood of six individuals who had scrub typhus, the organism was isolated after 1-18 months after the infection(29). The persistence of the organism needs further evaluation.

The vaccine development has failed multiple attempts for the past 70 years. The various approaches used were formalin-killed Orientia, inoculation of viable organisms followed by antimicrobial treatment, irradiated O. Tsutsugamushi and subunit vaccines(28)(30)(31). The results varied from short term to failure to protect. There has been failure to stimulate cross- protection against many strains in the nature after natural infection(32).

CLINICAL FEATURES

Scrub typhus is also known as the tsutsugamushi disease. It presents as an acute febrile disease. The illness varies from a mild and self limiting illness to a fatal disease. The case fatality rate of patients requiring admission in the hospitals was 4%(33).The incubation period varies from 6-21 days. The onset is characterised by fever, headache, myalgia, cough and gastrointestinal symptoms.

Classical course:

The classical description of the illness includes a primary papular lesion, which is the site of bite of the chigger. This site enlarges and undergoes central necrosis and crusts to form an eschar. The eschar is usually flattened and black in colour. About half of the patients develop non pruritic, macular or maculopapular rash, some patients also develop petechiae. The rash begins in the abdomen and spreads to the extremities. This is followed by regional lymphadenopathy and later generalised lymphadenopathy. The symptoms gradually increase in the severity with associated macular rash on the trunk. This leads to multiorgan involvement namely the central nervous system, respiratory, cardiovascular and gastrointestinal systems. Patients remain febrile for about 2 weeks and have a long convalescence of 4 to 6 weeks if left untreated(34).

Eschar:

Some patients develop a localised necrotic skin lesion at the site of the chigger bite. The eschar represents the site of inoculation where initial multiplication occurs before widespread dissemination. The eschar is usually painless and non-pruritic and usually

not reported by patients(35). The eschar may develop before the systemic symptoms and can occur in multiple locations. The frequency of the eschar can be variable. A study from Vietnam in 1973 showed a prevalence of 46%(36), sheehy et al showed an incidence of 60 to 88%(6). A study done in our institution in 2013, in 418 patients showed a distribution on the chest and abdomen (42.3%) in females, and in groin and genitalia (55.8%) in males. The unusual sites of the eschar are in the cheek, ear lobe and the feet(37).

Generalised lymphadenopathy is usually seen in majority of patients and usually accompanied by inflammation of lymphatic sinuses, splenomegaly and portal triaditis.

Relative bradycardia occurs commonly in scrub typhus, which is defined as a median decrease in heart rate <10beats/min per 1ºC increase in the temperature.

Complications of scrub typhus:

Scrub typhus involves multiple organs due to the microangiopathies leading to focal vasculitis or perivasculitis.

Respiratory system involvement

Pulmonary involvement is a well documented complication of the scrub typhus. The basic pathologic process in pulmonary involvement of scrub typhus is interstitial pneumonia with or without vasculitis(38).Pleural effusion is the most common radiographic feature with a prevalence of 12-55% in various studies(34). Cough occurred in up to 45% of the patients as shown by Berman et al(36). The other pulmonary manifestations are bronchitis, interstitial pneumonitis progresses to acute

respiratory distress syndrome, pneumonitis with patchy consolidation, pulmonary edema, cardiomegaly, hilar adenopathy, focal atelectasis, reticulonodular opacities, bronchial wall thickening and centrilobular nodules. Acute respiratory distress syndrome (ARDS) is defined as an acute and persistent lung inflammation with increased permeability has been reported in severe scrub typhus. The incidence of chest x-ray findings with scrub typhus varies between 59.4-78%(34). In a study done in Thailand 2006, 65% of patients had radiographic abnormalities(n=130), the most common abnormalities were bilateral reticular opacities(49%), congestive heart failure(19%)(39).

Cardiac disease:

Myocardial lesions were observed in about 80% in an autopsy series(40). Vasculitis and perivasculitis in the myocardium induce cellular infiltration along with haemorrhage and edema of interstitial tissues. Myocarditis is known to be associated with scrub typhus though incidence is not ascertained. IN critically ill patients scrub typhus patients cardiovascular abnormalities were seen in 61.7%(41).Cardiomegaly may be due to myocardial and pericardial involvement(38).

Renal involvement:

Renal abnormality has been reported in up to 82% of patients with scrub typhus. Acute kidney injury was reported in the 18-30% of cases, and in those requiring intensive care in up to 63.8%. Albuminuria and microscopic hematuria in 55-59% and 16-44%(41)(42).

Gastrointestinal and hepatic involvement:

Nausea, vomiting and diarrhoea are noted in one fourth of patients. Kim et al in 2000 showed 58% of patients with prominent gastrointestinal symptoms from 256 patients had superficial ulcers, erosions and actively bleeding ulcers(43). Abdominal pain is seen in 21.7% of those presenting with scrub typhus. Vomiting is seen in nearly 50% of patents and diarrhoea in 10-20% of cases. Acalculous cholecyctitis and pancreatitis progressing to pancreatic abscess has also been reported. Liver involvement is seen in over 60% of cases with scrub typhus. The common abnormalities noted are hypoalbuminemia, elevation of liver enzymes and alkaline phophatase and direct hyperbilirubinemia have been seen. Jaundice is seen in 132-22.2% of patients with scrub typhus. In a study done in our institution by chrispal et al showed a mean value of 2.1mg/dl (+ 2.4mg/dl). Severe hepatitis was not very common. The mean SGOT/SGPT values were 163.7 (±138.3) and 104.8 (±70.7) (U/L) respectively and serum ALP being 177.9(±127 U/L). Hypoalbuminemia has been seen in 68% of patient with scrub typhus in a study done by Razak et al. Add references.

Central nervous system (CNS) disease:

Scrub typhus is derived from the term "typho" meaning "stupor". The CNS is affected in 20-30% of patients infected by rickettsia. The CNS involvement in variable, the most common involvement is meningoencephalitis, other involvement includes encephalomyelitis. The various manifestations central nervous system are

1. Altered sensorium

Altered sensorium is seen as a part of encephalopathy in Scrub Typhus. A study done in Christian Medical College, Vellore by chrispal et al showed that the prevalence of altered sensorium was 22.2-32% in patients with scrub typhus(44). A study from Korea, the altered sensorium was seen in 2.6% of patients(45). Chrispal et al found altered sensorium to be an independent predictor of mortality(46).

2 Meningitis and encephalitis syndrome

Meningitis is a common CNS manifestation associated with Scrub Typhus. The symptoms noticed are nuchal rigidity and CSF analysis shows more than 5 cells/mm. Encephalitis is characterised clinically seizures and altered sensorium. There are few case reports of focal signs. The clinical syndrome may be mistaken for bacterial, viral or tubercular meningitis.

In a study from CMC, chrispal et al showed the prevalence of meningitis was 20.6%. Meningitis and meningoencephalitis presented with largely headache, neck stiffness, altered sensorium and seizures with CSF analysis suggestive of aseptic meningitis(46). In India the incidence of meningitis and meningoencephalitis ranged from 14 to 26%. Mahajan et al in 2006 reported a incidence of 21%(47), where as a study done in Karnataka showed an incidence of 20%(48). Vivekanandan et al reported aseptic meningitis in 14% of the 50 patients(49). In our institution a prospective study by chrispal et al showed the incidence to be 20.6%, while a retrospective study done by Varghese et al, in 623 patients showed incidence of 23.3%. Vishwanathan et al showed the incidence of 26% in patients with scrub typhus

meningitis. In a study done Kar et al 30% of cases presenting with acute encephalitis had scrub typhus.

3 Cranial nerve involvement: It is noted in scrub typhus to have cranial nerve involvement. The most well known cranial nerve involvement includes vestibulo cochlear nerve(1). The other nerves noted are optic, facial and abducens nerve(50)(51).

4 Cerebellar involvement:

Acute and subacute cerebellar involvement is noted in isolation or in combination with meningitis with scrub typhus. Cerebellar involvement is characterised by truncal ataxia, horizontal nystagmus with aseptic meningitis. In a series form 1991, scrub meningitis was associated with bilateral cerebellar signs in 72 of the patients(52). One case of cerebellitis was also noted in a series with scrub typhus by Razak et al(48).

5 Other central nervous system involvement:

Involvement of spinal cord is not very common with scrub typhus. There was a case by Kim et al of involvement of brain stem with associated bilateral facial and abducent palsy, with associated dysarthria and dysphagia in the 3rd week of the illness. Imaging (MRI) showed a non specific T2 hyperintense and T1 hyperintense lesions. It was also noted to have a gradual resolution with therapy(53). Acute disseminated encephalomyelitis (ADEM) is also reported in patients with scrub typhus with a rapid course and variable resolution with therapy. Transverse myelitis is also reported in patients with scrub typhus(54).

Peripheral nervous system involvement in the form of neuritis is noted with an incidence of 5.5%(55).Motor, sensory and mixed neuropathy are noted in patients with shoulder girdle most commonly involved. There are case reports of isolated brachial plexopathy after resolution of scrub typhus(56). Gullaine barre syndrome an ascending areflexic polyradiculopathy is noted with scrub typhus in few case reports(57). In India sawale et al noted a case of ascending flaccid quadriparesis and facial palsy in the convalescent period of the scrub typhus infection(58).

Extrapyramidal syndromes are also seen in scrub typhus. Acute parkinsonism was noted in 1946 by ripley et al(55). Movement abnormalities like tremors was noted in patients with scrub typhus meningitis by pai et al(59). Opsoclonus- myoclonus of a special clinical syndrome associated with scrub typhus, where there spontaneous ocular motility characterised by spontaneous, saccadic, arrhythmic and conjugate saccade without saccadic interval. Myoclonus is characterised by brief, shock like, involuntary movements caused by muscular contractions and inhibitions.

DIAGNOSIS

As in all the rickettsial infections no laboratory test is diagnostically reliable in the early phases of scrub typhus. The disease is recognised with compatible clinical symptoms, signs and laboratory features. Epidemiological factors aids in the diagnosis such as recent exposure to chiggers.

Laboratory parameters:

The common laboratory abnormalities noted in patients are thrombocytopenia which is associated with severe illness. Liver dysfunction in the form of elevation in the hepatic enzymes, bilirubin are seen. Renal failure leading to elevated creatinine is seen. They can have both leukocytosis, leucopoenia or in some instances the total leukocyte count can be in the normal range.

Definitive diagnosis

The diagnosis of scrub typhus can be definitively confirmed by four methods

1. **Serology :**

a) Indirect fluorescent antibody test (IFA) remains the mainstay of serologic diagnosis and is the gold standard for the diagnosis of scrub typhus. The diagnosis is confirmed by a fourfold increase in the titer from the samples drawn 14 days apart. The most commonly used strains for the test are the Kato, karp and Gilliam strains, and it uses fluorescent anti human antibody against the strains. It has been seen to have a large antigenic variability. The most common cut-off titer used is 1:50(5). It is more sensitive than the weil felix test(60).

b) ELISA (enzyme linked immunosorbant assay): ELISA has been developed which helps in rapid analysis of the sample. The 56kDA protein of the organism which is located on the rickettsial surface was cloned and was expressed as a fusion protein with maltose-binding protein of Escherichia coli by deleting the 252 base pairs from the 5'end. The recombinant protein was used for detection of the antibody in human sera. This yielded a sensitivity of 95% and specificity of 100%(61).

c) **Weil Felix test:** This was the oldest test which was in use, which was relatively inexpensive and easy to perform. However it lacks sensitivity and specificity.

2. Biopsy of eschar:

Eschar forms the pathological hallmark of scrub typhus. Damage to the endothelial cells occurs early in the infection and is evidenced by lymphohistiocytic vasculitis. Thus demonstration of the histological features of cutaneous necrosis, intense vasculitis with perivascular collections of lymphocytes and macrophages forms the hallmark of the disease. These can be demonstrated by fluorescent antibody conjugates even in the absence of rickettsia.

3. Culture:

Culture of this organism requires bio safety level-3 facilities and has to be cultured on a cell monolayer with median time to positivity being 27 days. Hence current methods of isolation of the organism are not appropriate for routine diagnosis of scrub typhus(60).

4. Polymerase chain reaction (PCR):

PCR can definitively establish the diagnosis of scrub typhus even in patients who lack IgM antibodies in the early course of the infection. The sensitivity of PCR was 85% and specificity was 100% in a study from Korea. In addition to serum PCR, eschar PCR is also sensitive and specific for the diagnosis(62). The PCR testing for 56kDA

antigen protein has been found to be highly specific. However the assay involving 16SrRNA showed a sensitivity of only 37.5-52.3%(60).

5. Loop isothermal amplification (LAMP):

It is a technique which is used to amplify the DNA using three specifically designed primer pairs and the Bst DNA polymerase. The entire reaction takes place at the same temperature. The reaction is read visually not requiring any special equipment. It is noted to detect as low as 14 copies/micltr compared to 3 copies/mic ltr in PCR(60).

The other tests which have been used are indirect immunoperoxidase (IIP), integrated diagnostic dip-stick test.

Table 3: Tests used for Scrub Typhus

Adapted from Gavin C.K.W.Koh et al 2010**(60).**

Format	Assay	Acute sensitivity	Specificity	Cost/sample	Time	Ease	Setting
Isolation	*In vitro* isolation (cell culture)[9]	+	+++++	+++++	7–60 days	+	BSL3 reference laboratory
Isolation	Mouse inoculation[10]	+	+++++	+++++	5–30 days	+	BSL3 reference laboratory
Serology	IFA[19]	++	+++	++++	2 hours	++	Reference laboratory/ hospital
Serology	IIP[3]	++	+++	+++	2 hours	++++	Reference laboratory/ Hospital
Serology	Weil–Felix OX-K[20]	+	++	+	6–18 hours	++++	Primary hospital
Serology	Rapid point-of-care tests (e.g., Integrated Diagnostics Dip-S-Ticks)[20]	++	+++	+++	< 30 minutes	+++++	Primary hospital
Genetic	Real-time PCR (16S, 56 kDa, 47 kDa, *groEL*)[13,15]	+++	+++++	+++	3 hours	+++	Reference laboratory/ hospital

MANAGEMENT

Scrub typhus is caused by a mite-borne infectious disease caused by Orientia tsutsugamushi. It is manifested by high fever, intense generalised headache, diffuse myalgia, associated with rash and eschar at the chigger bite. Scrub typhus lasts for 14 to 21 days without treatment. It may be associated with multiorgan system involvement, death may occur due to these complications. The patients treated with appropriate antibiotics become afebrile within 48 hours of starting therapy. Failure of defervescence within 48 hours is considered evidence for alternate diagnosis other than scrub typhus(6).

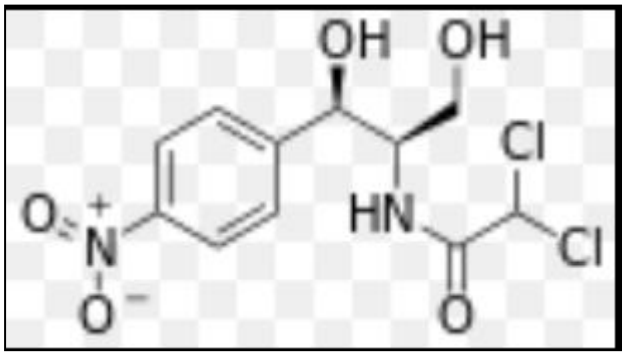
Antimicrobial therapy

There are various groups of antibiotics which have been tried in the therapy for scrub typhus.

1. **Chloramphenicol:**

Chloramphenicol is a bacteriostatic drug which acts by inhibiting protein synthesis. It acts by preventing the protein chain elongation by inhibiting the peptidyl transferase activity of the ribosome(63). It is known to specifically bind to the A2451 and A2452(64) residues of the 23S rRNA of the 50S ribosomal subunit thus preventing the peptide bond formation. It is very small molecule which is extremely lipid soluble and remains unbound to the protein. It penetrates effectively into all the tissues of the body including the brain.

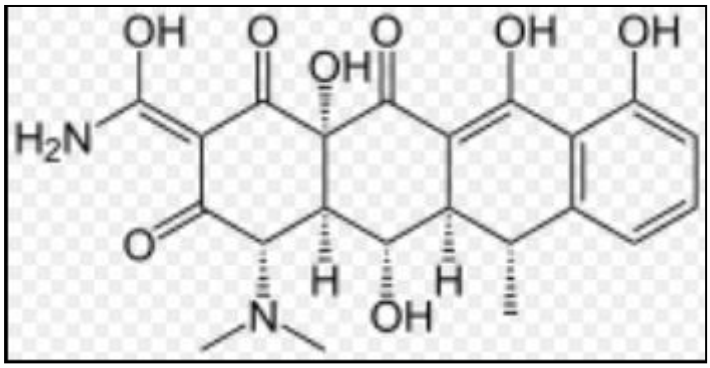
Figure 4: Chloramphenicol molecule



2. **Doxycycline and tetracycline:**

It is a broad spectrum antibiotic of the tetracycline class. It is useful against bacterial, protozoal and helmithic organisms and rickettsial infections(65). Doxycycline- metal ions are stable in the acidic pH, hence it is absorbed in the duodenum (66). The drug acts by entering the bacterial cell and reversibly binding to the 30s ribosomal subunit at the position blocking the binding site of the aminoacyl-tRNA acceptor site. This leads to inhibition of the protein synthesis(67).

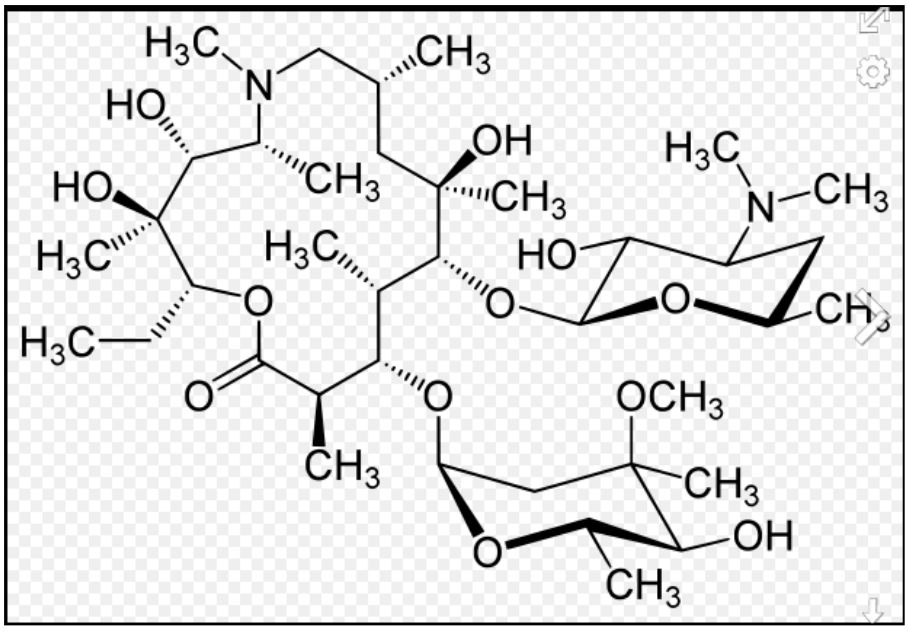
Figure 5: Doxycycline molecule



3. Azithromycin:

Azithromycin belongs to the azalide class which is a subclass of macrolides antibiotic class. It is derived from erythromycin with a methyl substituted nitrogen atom into the lactone ring. This makes Azithromycin a 15 member ring. It has a broad but shallow spectrum of antibacterial activity. It inhibits gram positive, gram negative and also atypical bacteria(68). It acts by interfering the protein synthesis by binding to the 50S ribosome unit and inhibiting the translation of mRNA. It is an acid stable antibiotic, hence facilitating oral intake of the drug. The drug gets concentrated in the phagocytes hence it gets actively transported to the sites of the infection. It has a long half life hence allowing a single dose therapy. However the nucleic acid synthesis is not affected by the drug(69). It has been used successfully in treatment of scrub typhus and other Rickettsial infections.

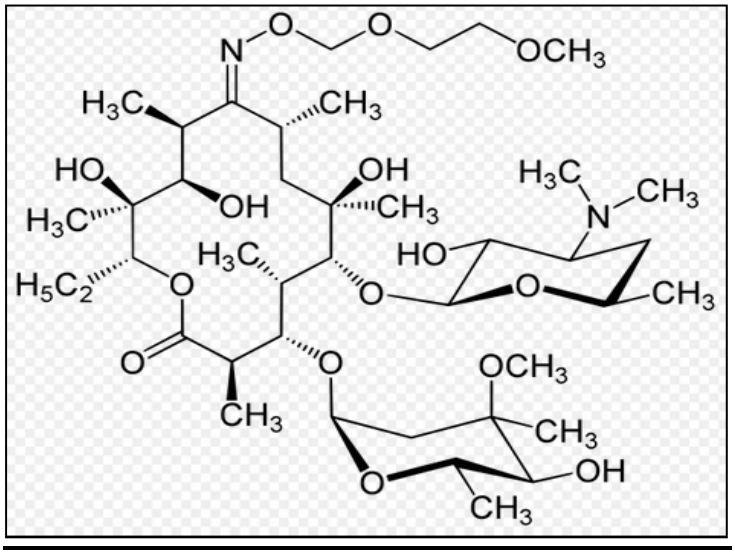
Figure 6: Azithromycin- molecule



4. **Roxithromycin**:

It is a semi-synthetic macrolides antibiotic which is also derived from the erythromycin antibiotic. It comprises of a membered lactone ring. It binds to the 50S ribosome and thus inhibiting the translocation of the peptides. It has similar antimicrobial spectrum as compared to erythromycin(70). There are various trials using roxithromycin for treatment of rickettsial infections.

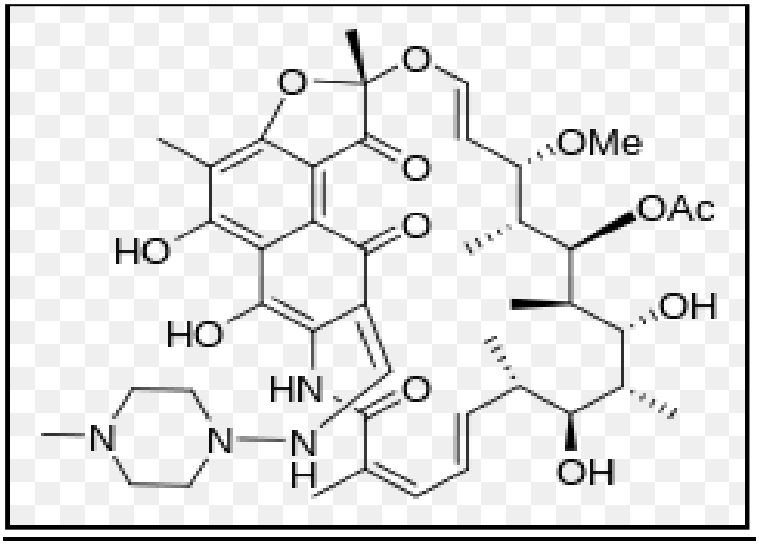
Figure 7: Roxithromycin molecule



5. **Rifampicin:**

It is a bactericidal antibiotic of the rifamycin group. It acts by inhibiting the bacterial DNA- dependent RNA synthesis by inhibiting the DNA- dependent RNA polymerase(71)(72). It can be used in variety of infection; it has been tried in patients with infection with rickettsial infections.

Figure 8: Rifampicin molecule:



Evidence for antibiotic usage in scrub typhus

Chloramphenicol was the first drug to be shown effective against scrub typhus and is commonly in endemic regions. Sheehy et al in 1973 included 63 participants, in which 30 received Chloramphenicol and the rest tetracycline with the outcome looked at was duration of fever and afebrile after 48 hours. It was found that the mean duration of fever in tetracycline arm was 28 hours and in Chloramphenicol arm was 35 hours, relapse was noted in 2 participants in the tetracycline arm and 5 in the Chloramphenicol arm(6)(73).

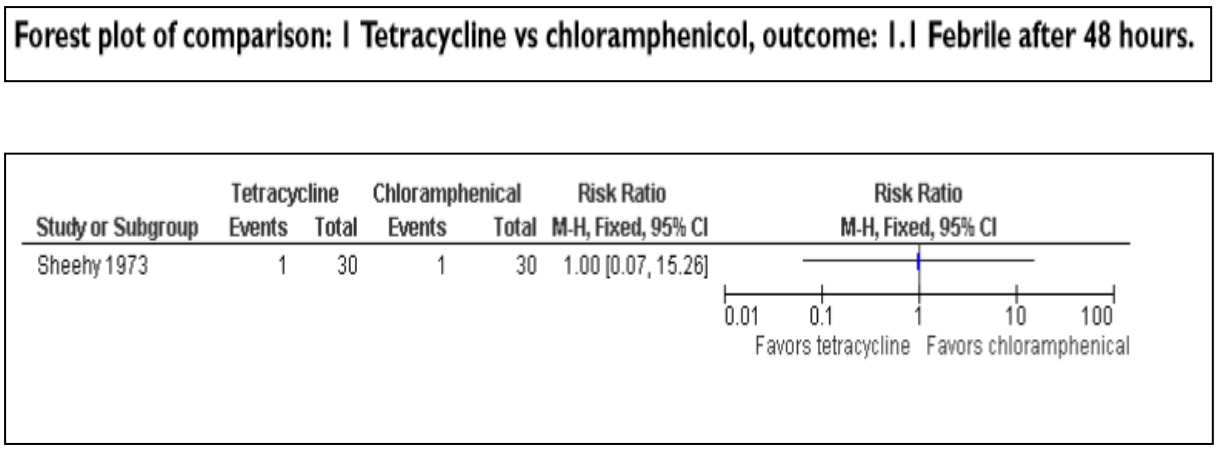


Figure 9: Adapted from Sheehy et al(6). Forest plot of comparison of fever, tetracycline v/s Chloramphenicol

There are five trials comparing Azithromycin and Chloramphenicol. There were 4 studies which compared duration of fever, symptom clearance and all the 5 studies compared treatment failure between the two groups. Li yuan et al 2004 included 136 subjects and the outcomes considered were adverse effects, fever clearance time, cure and disappearance of main symptoms(74). Wei et al included 62 subjects and they looked at cure and adverse effects(75). Wu xiang et al in 2006 looked at cure and disappearance of main symptoms including 62 subjects(76). Li et al in 2007 looked at fever clearance time, cure, disappearances of main symptom and adverse drug effect, studying 20 subjects(77). Chen et al studied 279 subjects to study fever clearance time, cure and adverse effects(78). Metaanalysis done showed Q test being significant for heterogeneity for duration of fever and symptom among the above said trials. The mean duration of fever clearance time was higher in Azithromycin arm than in the Chloramphenicol arm with a mean difference of 12.66 hours. However there was no

difference in the two arms in perspective to symptom clearance time and treatment failure(79).

Figure 10: Adapted from Fang y et al(79).Forest plot for duration of fever between Azithromycin and Chloramphenicol.

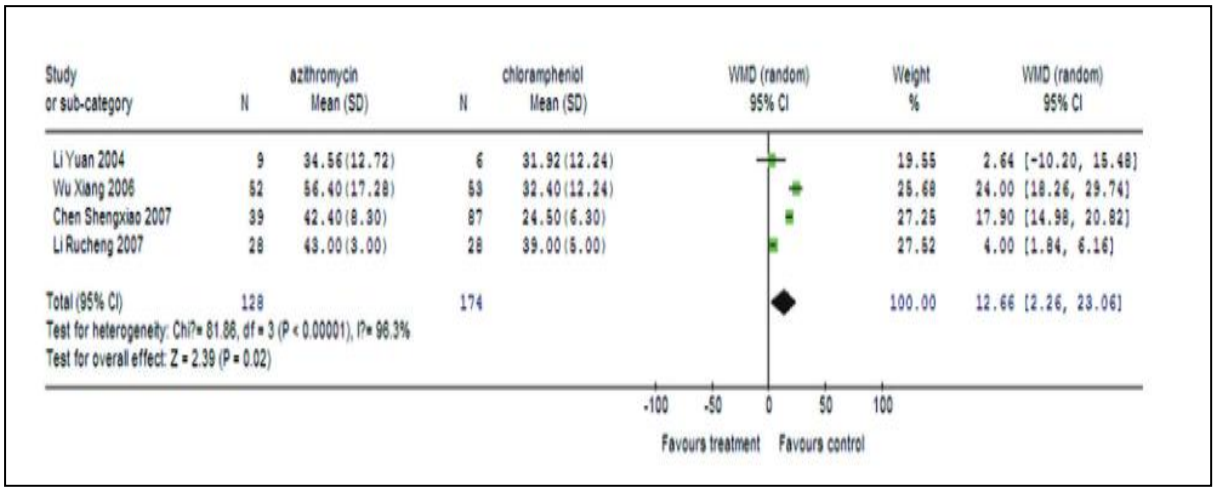
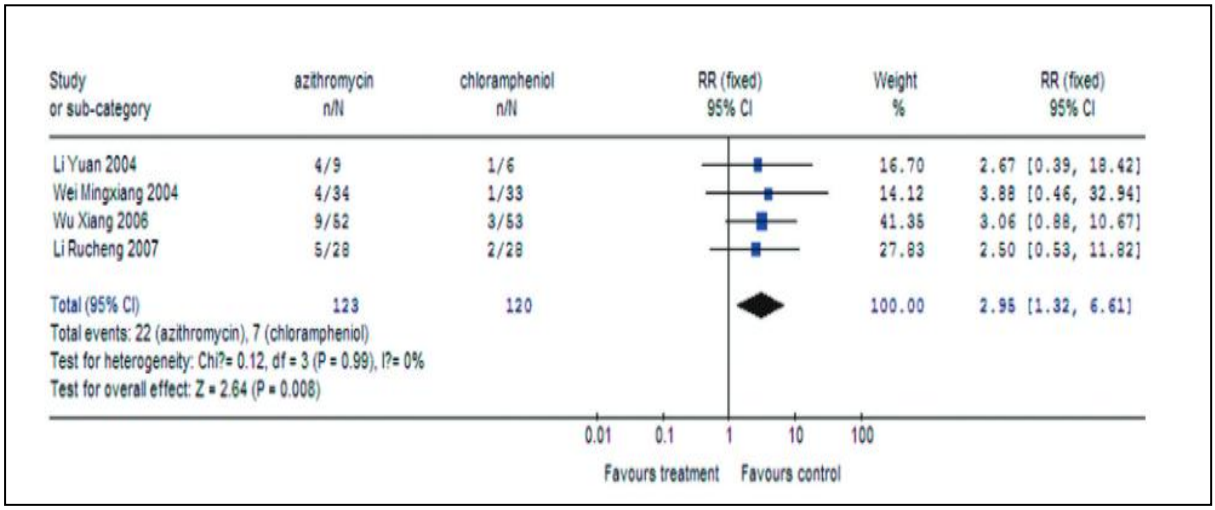


Figure 11: Adapted from Fang y et al(79). Forest plot for adverse effects between Azithromycin and Chloramphenicol



There are six trials compared doxycycline and Chloramphenicol. Li yuan et al 2004 included 136 subjects and the outcomes considered were adverse effects, fever clearance time, cure and disappearance of main symptoms(74). Yang et al in 2005, compared cure and disappearances of main symptoms(80). Wu xiang et al in 2006 looked at cure and disappearance of main symptoms including 62 subjects(76). Li et al in 2007 looked at fever clearance time, cure, disappearances of main symptom and adverse drug effect, studying 20 subjects(77).Feng et al included 210 subjects, compared cure and disappearance of symptoms(81). Phimda et al looked at 57 subjects and studied cure failure, defervescence and adverse events(82). Main symptom clearance time in days was shorter in doxycycline treated group compared to Chloramphenicol group with a mean difference of -0.4 days(79).

Figure 12: Adapted from Fang y et al(79).Forest plot for main symptom clearance between Doxycycline and Chloramphenicol.

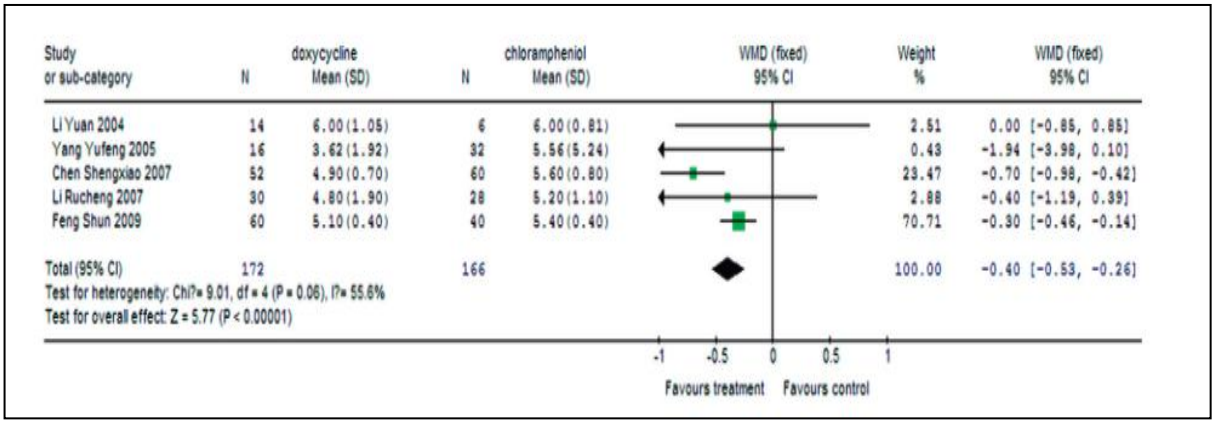
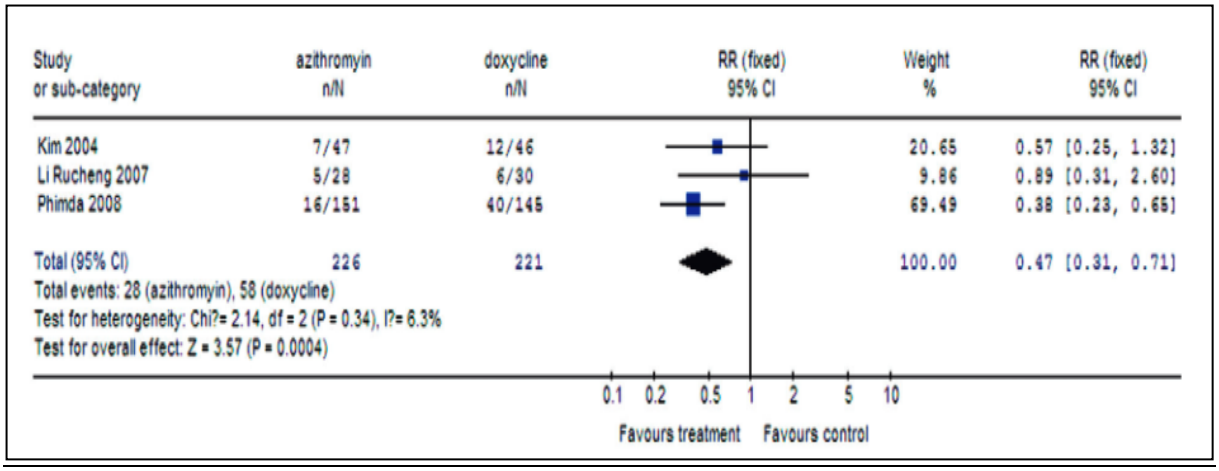
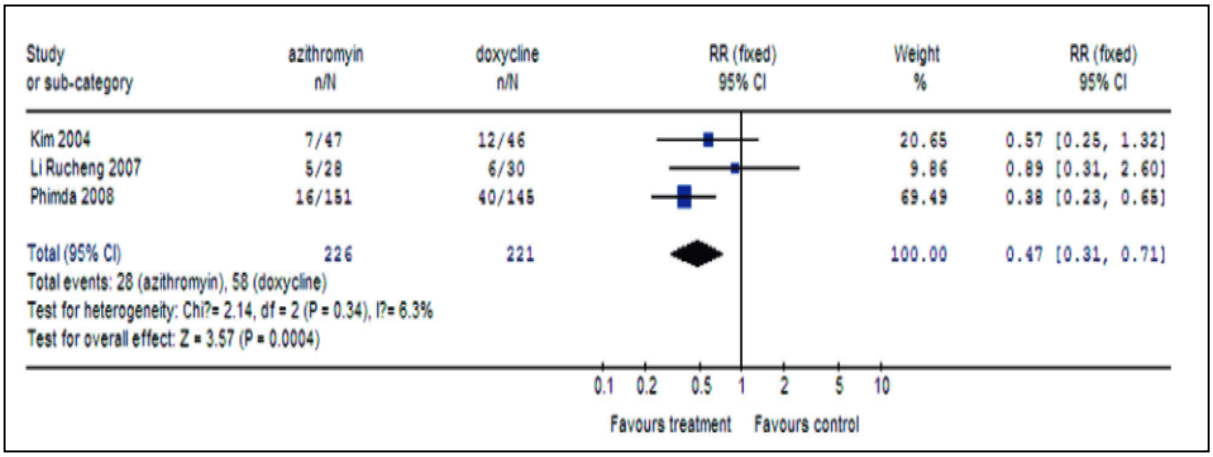


Figure 13: Adapted from Fang y et al(79).Forest plot for adverse events between Doxycycline and Chloramphenicol.



Azithromycin and doxycycline was studied in three randomised trials. Phimda et al looked at 57 subjects and studied cure failure, defervescence and adverse events(82). Li et al in 2007 looked at fever clearance time, cure, disappearances of main symptom and adverse drug effect, studying 20 subjects(77). Kim et al included 93 and looked at time to fever defervescence, cure, failure, adverse effects(7). There was no difference detected in duration of fever, symptom clearance time and treatment failure. However the adverse events were less likely to occur in Azithromycin treated group(79).

Figure 14: Adapted from Fang y et al(79).Forest plot for adverse events between Doxycycline and Azithromycin.



Three trials compared roxithromycin and doxycycline which showed no difference in terms of treatment failure and symptom clearance time. Two trials compared rifampicin and doxycycline which had no difference for the event, or adverse events. There was no difference between tetracycline and doxycycline(79).

Treatment with doxycycline showed rapid reduction of clinical manifestations, oral doxycycline is the currently the standard of treatment for mild cases(82). Azithromycin is reported to be equally effective as doxycycline for the treatment of scrub typhus, with lesser gastrointestinal symptoms(73)(7).

A study done in three centres in Thailand by K Phimda et al in 2014,included 296 patients of which 69(23.3%) had leptospirosis, 57(19.3%) with scrub typhus and 14(4.7%) with murine typhus and 11 (3.7%) with co-infection with leptospirosis and rickettsial infection. The study compared Azithromycin and doxycycline, the results showed that the overall cure rate of Azithromycin (97.4%) was non-inferior to doxycycline (96.5%) treated arm. Among patients with laboratory confirmed scrub typhus, treatment failure occurred in 1 patient, the median time of fever defervescence

was 48 hour in doxycycline group and 60hour in Azithromycin group. However doxycycline showed earlier defervescence than Azithromycin group(83).

FEVER DEFERVESCENCE

It has been seen that there is a delayed defervescence in fever in patients with appropriate therapy for scrub typhus. Chung et al, 2008 studied 130 cases, which had 38 patients with scrub typhus, 61 with Q fever and 7 with murine typhus. All the patients received 200mg orally in two divided doses per day of doxycycline. 7 patients with scrub typhus had delayed defervescence. Relative bradycardia, jaundice and absence of headache were associated with delayed defervescence. The postulate for delayed defervescence is the probable presence of resistant strains to doxycycline(8).

All the studies thus far have compared antibiotics and have shown to have similar results with therapy. There is lack of studies looking at antibiotic profile in patients with severe scrub typhus disease and the resolution of the organ failure. Role of combination therapy in scrub typhus hasn't been studied in terms of non inferiority or superiority to monotherapy. The proposed study aimed at studying the shortcomings of the earlier studies.

MATERIALS AND METHODS

Study Setting:

The study was conducted in Christian Medical College, Vellore. This is a 2700 bedded academic medical centre providing tertiary medical care to the residents of Vellore and surrounding districts of Tamil Nadu and Andhra Pradesh. It is also a referral care centre for patients from other parts of India and South East Asia.

Duration: The study was conducted between November 2013 and January 2015. As Scrub Typhus is a seasonal disease which is more prevalent in the cooler months of the year, two seasons were included in the study.

Study Design:

This was a prospective observational cohort study.

Participants:

The study included all patients older than 18 year of age with acute undifferentiated febrile illness with a diagnosis of Scrub Typhus presenting to the hospital to Outpatient department, Intensive care unit /High dependency unit/Medical wards.

We recruited a total of 223 patients from November 2013 to January 2015.

Inclusion Criteria:

All adult patients aged ≥ 18 years of age presenting with an acute undifferentiated febrile illness, with clinical profile suggestive of scrub typhus with.

1. Positive Scrub Typhus IgM Elisa and/or
2. Presence of a characteristic eschar.

Exclusion criteria:

1. Patients with acute febrile illness with a definitive etiological diagnosis other than scrub typhus.
2. Patients on immunosuppressive agents or known to be immunocompromised.
3. Patients with a known autoimmune disorder.
4. Patients with a known malignancy on chemotherapy.

Rationale for Diagnosis and Inclusion:

An eschar correlated with a serological positivity of 99% of cases suspected to have scrub typhus. In addition our experience has shown good clinical response to therapy for scrub typhus in those presenting with appropriate clinical symptoms and an eschar. Hence in our setting, an eschar was considered diagnostic of scrub typhus.

Serological testing was done using Scrub Typhus Detect IgM ELISA (InBios). Positivity was indicated with titers more than or equal to 0.5 OD. This kit uses a 56-kDa recombinant cocktail of antigens (Karp, Gilliam, Kato and TA763). It has 86.5% sensitivity and 97.5% specificity.

Patients with active of past history of autoimmune disease, immunosuppression and malignancies on chemotherapy which could have a probable confounding effect on the defervescence pattern were excluded from the study.

Methodology:

A single centre observational prospective cohort study was done to determine the factors associated with outcome of scrub typhus between November 2014 to february 2015.A written informed consent was taken from either the patient, or from the legal guardian in those with altered mental status, prior to the enrollment. All patients fulfilling the inclusion criteria were recruited within 24 hours of the presentation to the hospital. All patients with a positive scrub typhus IgM and or a characteristic eschar were included for the study and further analysis.

A study form/data abstraction sheet which included patient's demographic details, co-morbidities, and clinical features was noted (attached as annexure). The details of antibiotics used by the treating physician including the time of administration, duration and the types of antibiotics used were recorded and the choice was not influenced by the study. In this centre three regimens are in practice namely doxycycline group, azithromycin group and the combined group receiving

doxycycline and azithromycin. Azithromycin is preferred in children and pregnant women, however the combination is more commonly used in the severely ill subjects as oral doxycycline absorption is suspected to be erratic in this group.

Severity of the acute illness was assessed by using sequential organ failure assessment (SOFA) score was used which was repeated three times in patients getting admitted to critical care units. These were assessed within 48 hours after the admission and sequentially after 48 hours. The SOFA score is a standardized score used to assess the severity of the illness; it includes the P/F (PaO2/FiO2) Ratio, the GCS (Glasgow coma scale), total bilirubin, platelets, creatinine with increasing score indicating increasing severity of the illness. It has a direct correlation with the mortality as the outcome.

The outcomes assessed were

Primary**:**

The incidence of delayed defervescence in patients treated with azithromycin, doxycycline and the combination of the two.

Secondary:

a. Factors associated with delayed defervescence.
b. Duration of organ recovery.
c. Comparison between the antibiotic regimens with respect to time of defervescence across the 3 arms.

Definitions

The time of defervescence will be defined as the first day of the highest body temperature lower than 37.7°C (100°F) for more than 3 consecutive days without the use of antipyretics.

Multi-organ dysfunction: Involvement of any 2 of the following organs was considered as having dysfunction (4).

a. Respiratory dysfunction: SAO2<90% or PaO2/FiO2 ratio< 300 or need for ventilator assistance;
b. Renal dysfunction: Serum Creatinine > 2mg/dl or need for dialysis;
c. Cardiovascular dysfunction: Hypotension(<80/60mmhg) or need for inotropes or vasopressor support;
d. Hepatic dysfunction: Serum Bilirubin >2mg/dl or three fold elevation of liver enzymes;
e. Neurologic dysfunction: Alteration in the level of consciousness.

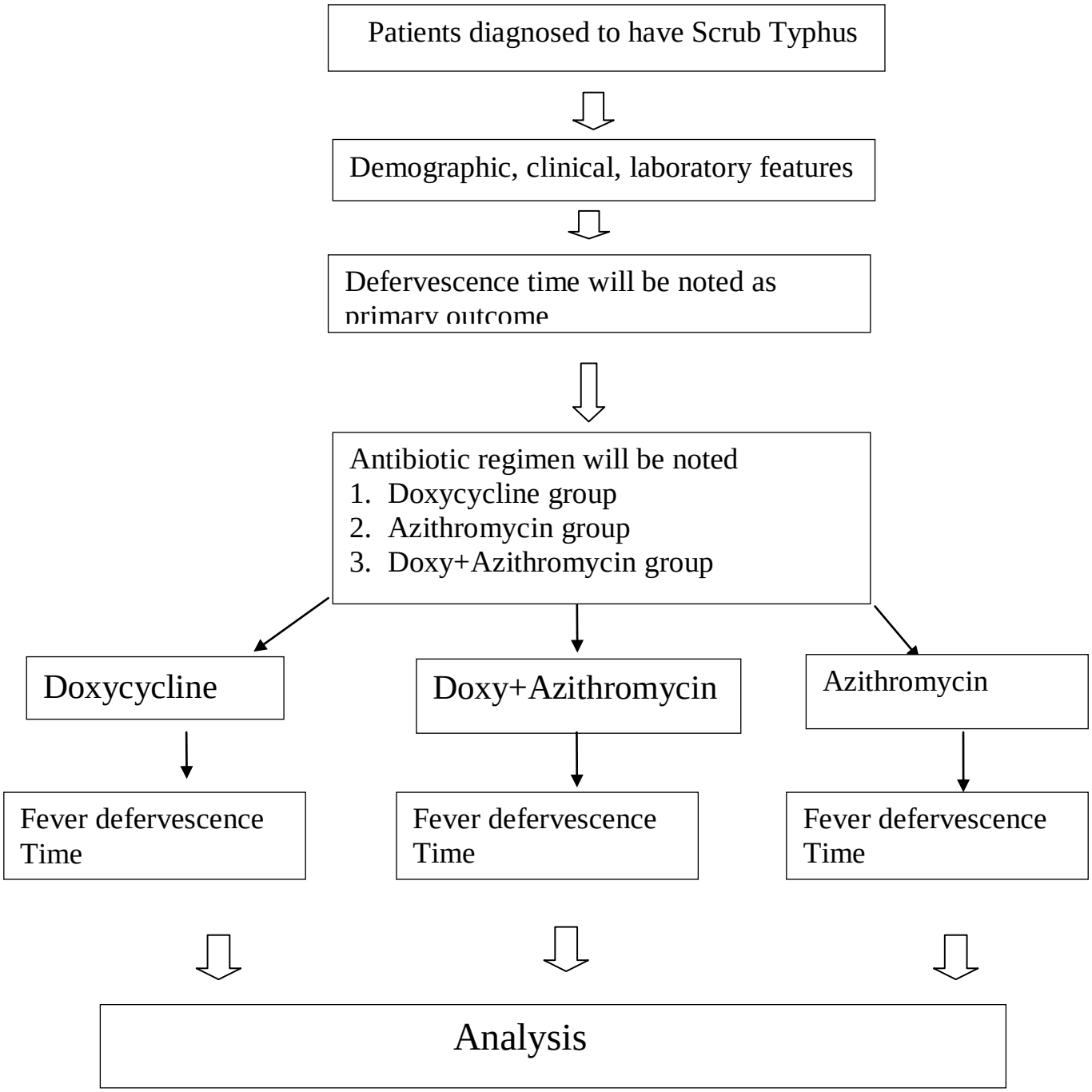
Exposure: All with diagnosed scrub typhus infection (as per case definition)

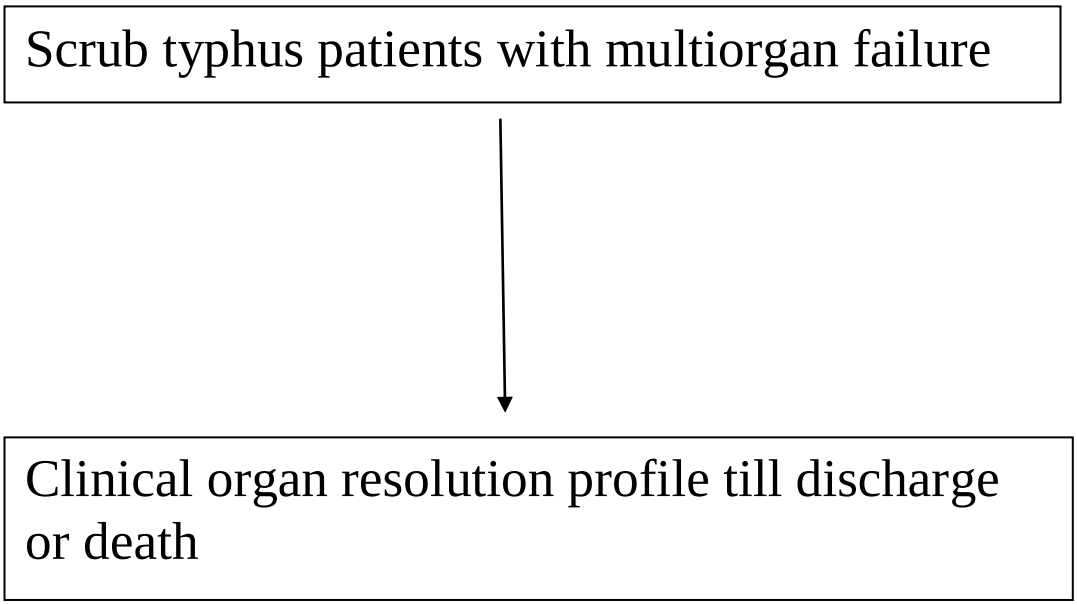
Potential confounders:

a. Use of antipyretics and analgesics
b. Spontaneous resolution of fever without antibiotic treatment
c. Steroid use.

Follow up: The patients were followed up for a period of 2 weeks after recruitment. If discharged earlier than 2 weeks a telephonic call was made to determine the outcomes.

STROBE STATEMENT- STUDY FLOW CHART





1. **Sample size**

The sample size was calculated based on the primary outcome which is finding the incidence of delayed defervescence in all scrub typhus patients who have been treated with Azithromycin, doxycycline and the combination of the two. The sample is sufficiently large to compare the three arms.

Single Proportion - Absolute Precision

Expected proportion	**0.21**
Precision (%)	**5**
Desired confidence level (1- alpha) %	**95**
Required sample size	**255**

The sample size to show an incidence of 21.5% of delayed defervescence with a Precision of 5% was found to be 255 subjects with 95% confidence limits.

Formula:

$n = Z*PQ/d^2$

Where

Z = 95% confidence limits

d = precision of 5%

P = incidence of 21%

Reference :

Kim YS, Yun HJ, Shim SK, Koo SH, KimSY, Kim S. A Comparative trial of a single dose of azithromycin versus doxycycline for the treatment of mild scrub typhus. Clinical Infectious Diseases 2004; **39:1329**–35. Where incidence was found to be 21%.

STATISTICAL METHODS

Data entry was done using the Microsoft excel software and analysis was done using Statistical Package for the Social Sciences (SPSS) software package (version 15). Descriptive statistics were calculated using SPSS software. Chi-square test was used for comparison of categorical variables. Odds ratio (OR) and confidence intervals (CI) were calculated and a '*p*' value less than 0.05 was considered statistically significant.

All reported *p* values are two-sided. Univariate analysis such as Independent t-test and Mann Whitney U test, Kruskall Wallis tests were performed to assess the risk factors for clinical outcome among the study patients. Logistic regression was done to look for combined significance of the variables.

Ethics committee clearance and funding:

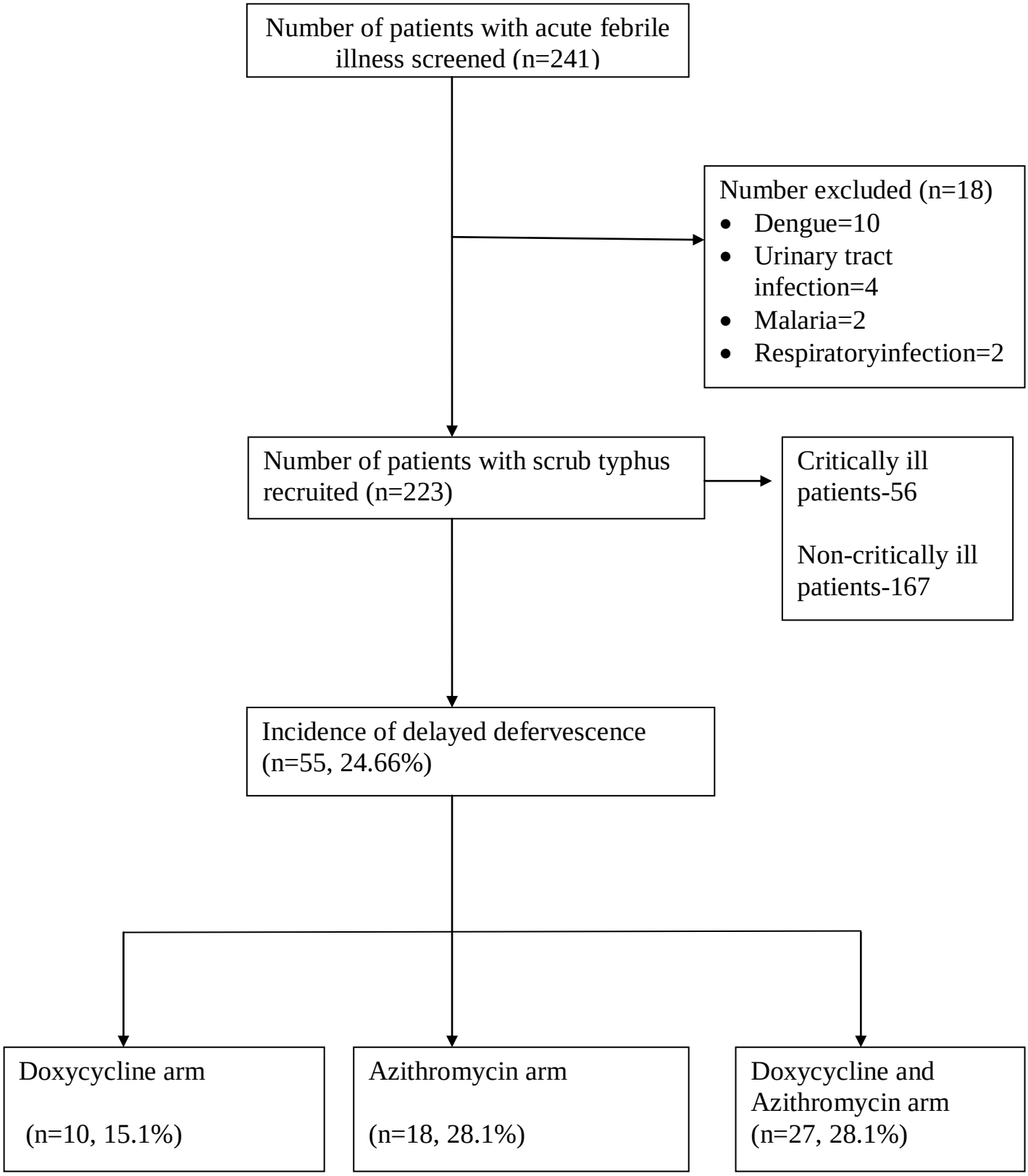
The study design and methods were approved by the Fluid Research Committee, Christian Medical College Vellore.

RESULTS

This prospective observational cohort study was conducted from November 2013 to January 2015. During this period 241 patients were screened for scrub typhus, of which eighteen were excluded. Ten of them had dengue, four had urinary tract infection, two had malaria and two had respiratory tract infection.

Two hundred and twenty three (n=223) satisfied the inclusion criteria for scrub typhus and were included in the study (strobe statement-figure 15).

Figure 15: STROBE STATEMENT-STUDY FLOW CHART



BASELINE CHARACTERISTICS

Demographic features of patients with scrub typhus

Table 5: Demographic features of patients with scrub typhus(N=223)		
Variable	**Frequency(N=223)**	**Percentage (%)**
Demographics		
Mean age in years, Mean (SD)	47.6 (15.1)	
Sex		
Male	102	45.7%
Female	121	54.3%
Occupation		
House wife	119	53.4%
Manual labourer	49	22%
Farmer	37	16.6%
Student	9	4%
Business	9	4%
State		
Tamil Nadu	165	74%
Andhra Pradesh	54	24.2%
West Bengal	3	1.3%
Karnataka	1	0.4%

- **Demographic characteristics**

The study cohort comprised of 223 patients with the most common age group of 40-60 years (figure 2), mean age 47.6 ±15.12years. There was female preponderance with 54.3% females and 45.7% males. The patients were predominantly from Tamil Nadu 74% (n=165) followed by 24.2% from Andhra Pradesh, 1.3% were from west Bengal and 0.5% from Karnataka (figure 16).

The majority of subjects were house wives 53.4%, followed by manual laborers (22%), farmers (16.6%), businessmen and students were 4% each (Table 5).

- **Co-morbidities**

The predominant co-morbidity was diabetes mellitus which was seen in 10.3% (n=23) followed by hypertension (9%), cerebrovascular accident (0.9%), ischemic heart disease (0.9%), chronic liver disease (1.3%), alcoholism (2.7%), COPD (0.9%) and pregnancy (2.7%).

Figure 16: Age distribution in patients with scrub typhus (N-223)

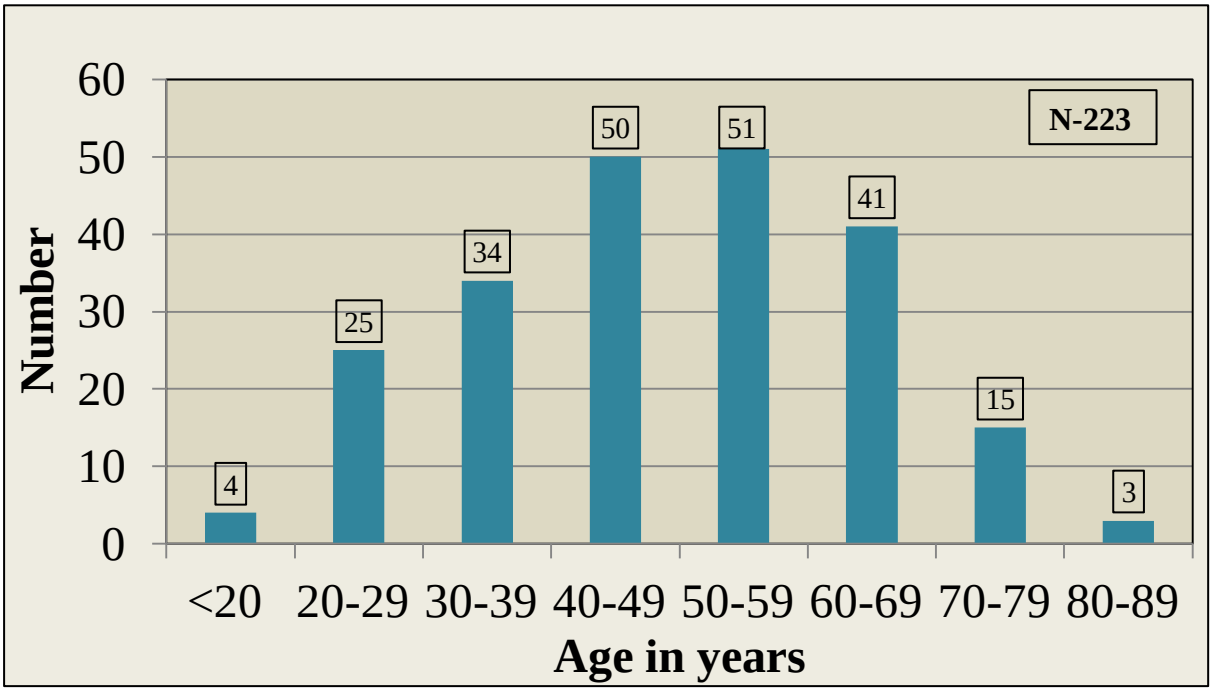


Figure 17: Gender distribution in patients with scrub typhus (N-223)

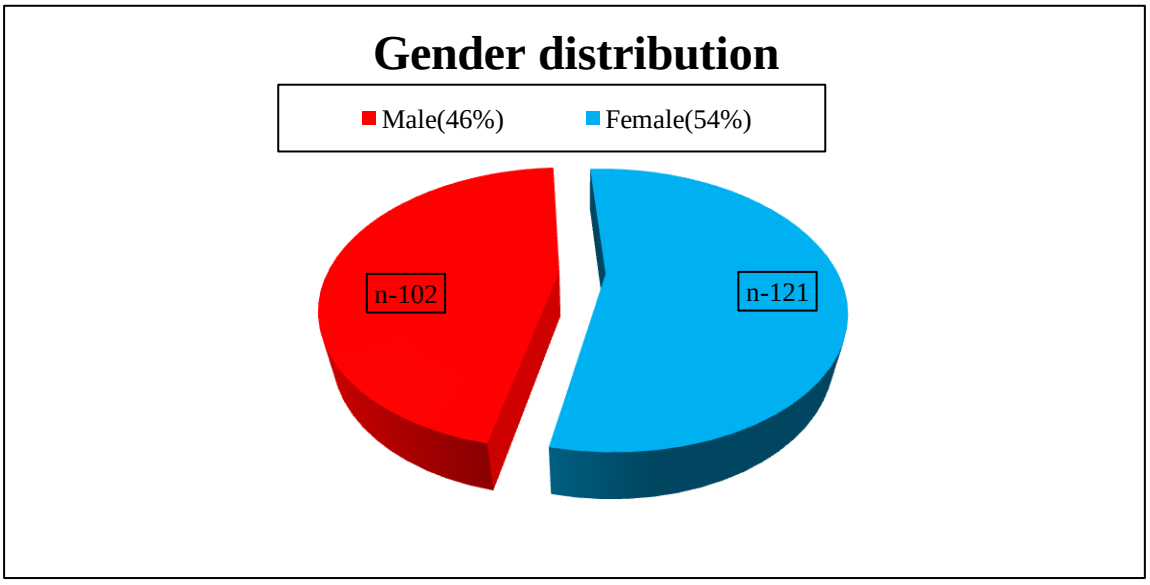


Figure 18: State wise distribution in patients with scrub typhus (n-223)

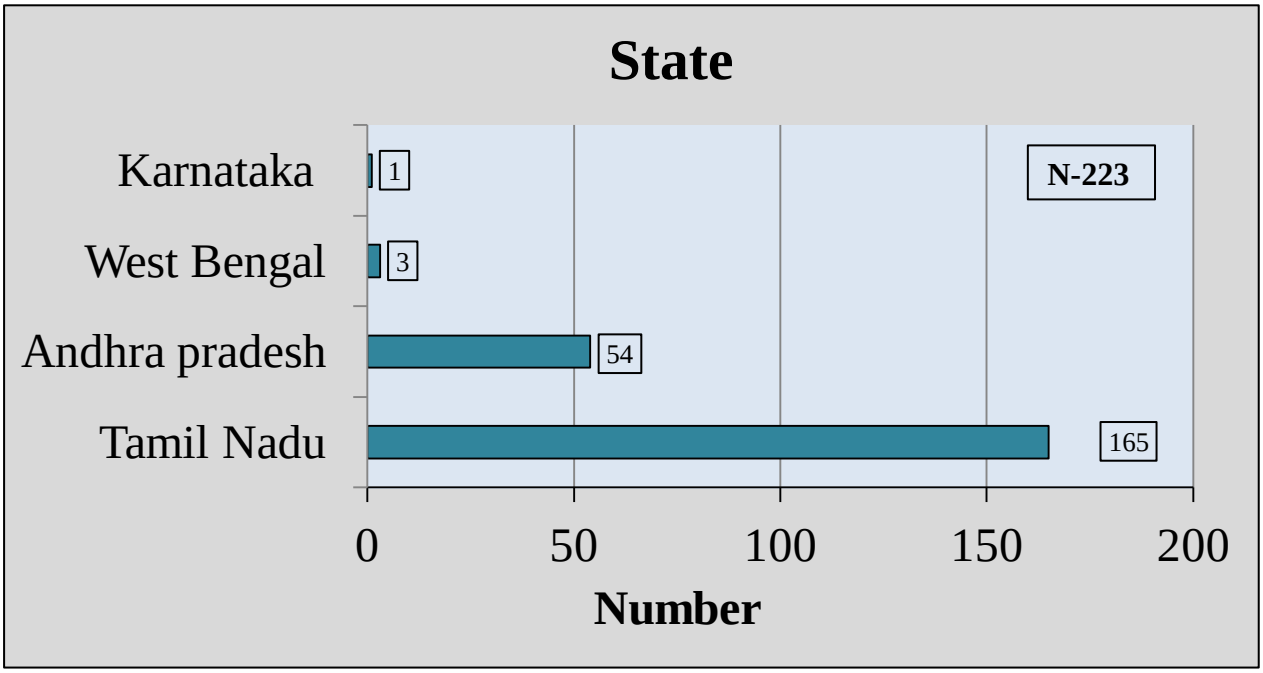


Figure 19: Occupation distribution of patients with scrub typhus (N-223)

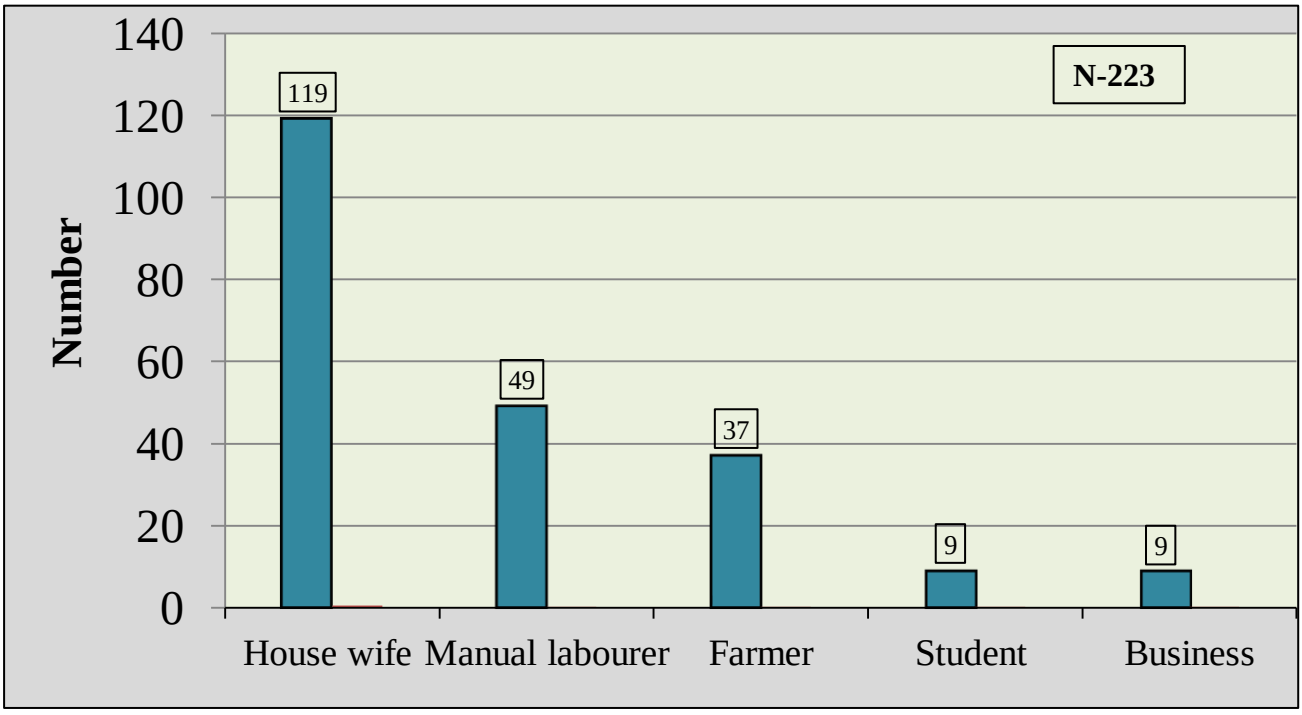


Table 4: Clinical characteristics in patients with scrub typhus

Table 4:Clinical features in patients with scrub typhus (N=223)	
Symptoms	**Frequency n (%)**
Chills	216 (96.9)
Breathlessness	197 (88.3)
Myalgia	121 (54.7)
Cough	99 (44.4)
Headache	64 (28.7)
Altered Sensorium	64 (28.7)
Vomiting	48 (21.5)
Seizures	6 (2.7)
Bleeding	5 (2.2)

- **Clinical signs in patients with Scrub Typhus (N=223)**

Table 5:Clinical signs in patients with Scrub Typhus (N=223)	
Signs	**Frequency**
Pulse, Mean ±SD	109.3 ± 19.59
Systolic BP, Mean ±SD	105 ± 19.76
Diastolic BP Mean ±SD	66.5 ± 12.55
Respiratory rate, Mean ±SD	31.5 ± 10.30
Crepitations, n (%)	183 (82.1)
Eschar Positive, n (%)	161 (72.2)
Desaturation, n (%)	112 (50.2)
Hepatomegaly, n (%)	14 (6.3)
Splenomegaly, n (%)	2 (0.9)

All the patients in this cohort had fever as the presenting complaint, followed by breathlessness in 88.3%, myalgia (54.7%), cough (44.4%), headache (28.7%), altered sensorium (28.7%), vomiting (21.5%) and bleeding and seizures in 2.2% and 2.7% of patients respectively (Table 4).

The mean pulse rate was 109.3 ± 19.59 per minute; the systolic and diastolic blood pressures were 105 ±19.76 mm of hg, 66.5 ±12.55 mm of hg respectively. Eschar was seen in 72.2% of the patients, with the most common site being axilla (n=45, 17.5%) followed by back (13.5%), groin (11.7%), chest (9.9%), abdomen (5.8%) and trunk (5.8%, Figure 1). The predominant sign was crepitation which was seen in 82.1% of the subjects followed by desaturation (50.2%), hepatomegaly (6.3%), and splenomegaly (0.9%) (Table 5)

Figure 20: Presence of Eschar in patients with Scrub Typhus (N=223)

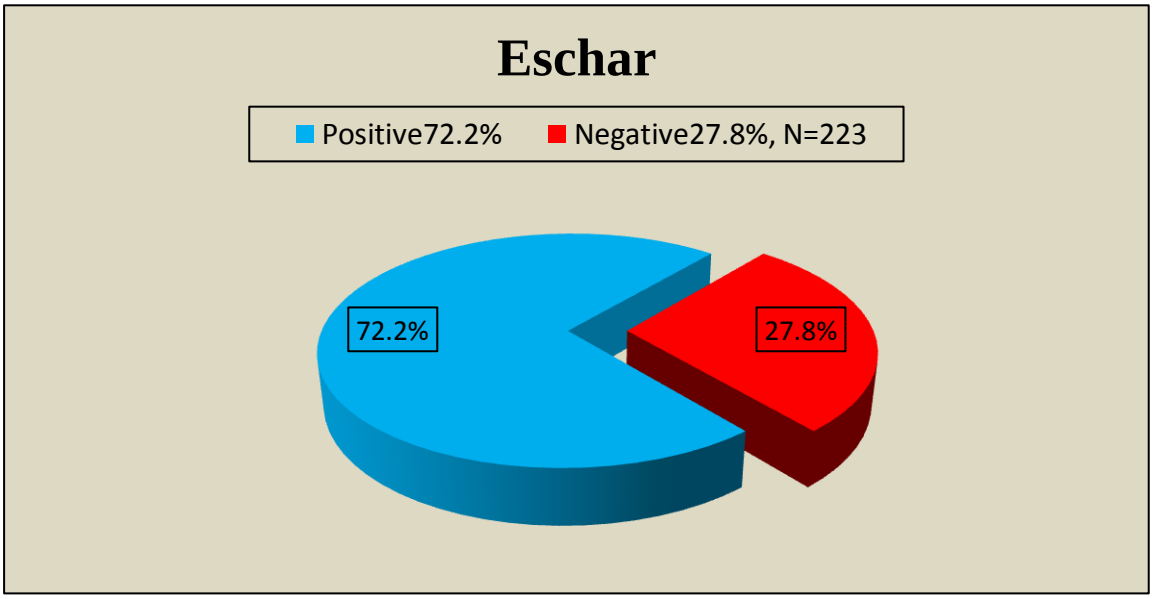
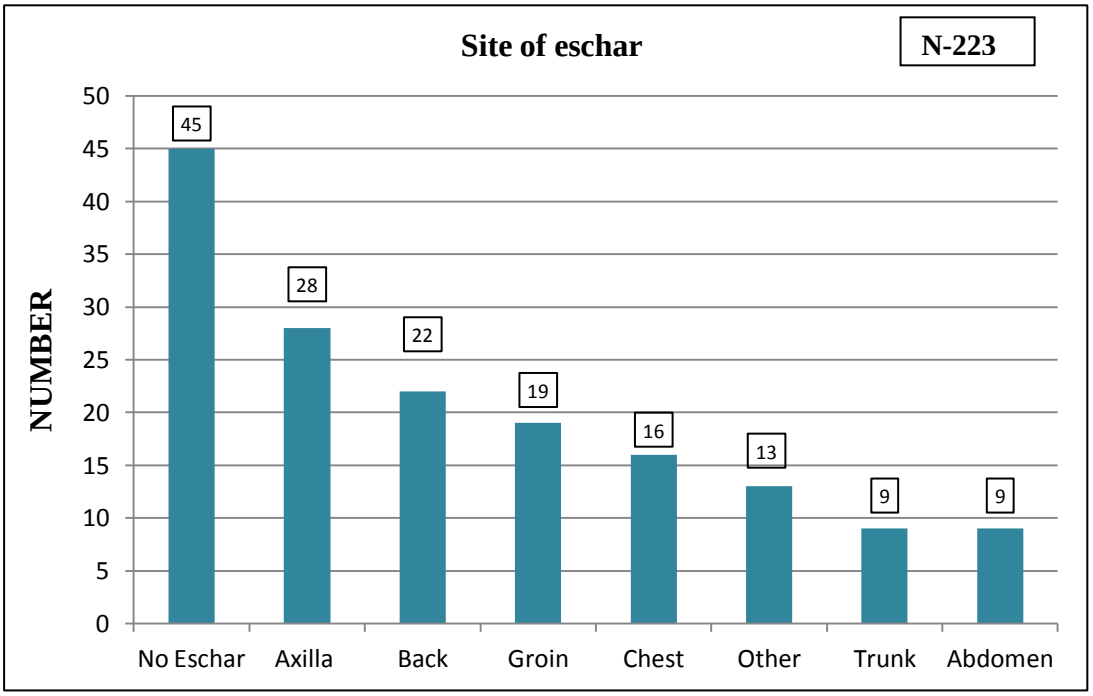


Figure 21: Site of Eschar in patients with Scrub Typhus (N=223)



- **Baseline characteristics in the three antibiotic arms**

Table 6:Baseline characteristics between the three antibiotic arms (N=223)				
Variable	**Doxycycline group (n=63), n(%)**	**Azithromycin group (n=64), n(%)**	**Doxycycline + Azithromycin group (n=96) n(%)**	***P* value**
Demographics				
Male	36(57.2%)	26(40.6%)	40(41.6%)	0.099
Female	27(42.8%)	38(59.4%)	56(58.4%)	
Clinical Features				
Duration of illness, days, mean(SD)	8.09(3.6)	8.75(4.5)	8.21(4)	0.104
Chills	61(96.8%)	64(100%)	91(94.8%)	0.180
Breathlessnes	46(73%)	58(90.6%)	93(96.8%)	<0.001
Myalgia	43(68.2%)	30(46.9%)	48(50%)	0.029
Cough	26(41.2%)	31(48.4%)	42(43.7%)	0.709
Headache	24(38%)	15(23.4%)	25(26%)	0.141
Vomiting	12(19%)	13(20.3%)	23(23.9%)	0.733
Abdomen pain	8(12.7%)	7(10.9%)	12(12.5%)	0.943
Seizures	1(1.58%)	1(1.5%)	4(4.1%)	0.496
Co-morbities				

Table 6- Baseline characteristics between the three antibiotic arms (continued)				
Variable	**Doxycycline group (n=63), n(%)**	**Azithromycin group (n=64), n(%)**	**Doxycycline + Azithromycin group (n=96) n(%)**	***P* value**
Diabetes Mellitus	8(12.7%)	5(7.8%)	10(10.4%)	0.731
Hypertension	5(7.9%)	6(9.3%)	9(9.3%)	0.944
Pregnancy	0	5(7.8%)	1(1.04%)	0.010

In this cohort, there was an increased proportion of males in doxycycline arm (57.2%), more females in azithromycin arm (59.4%).

The duration of fever at presentation was least in the doxycycline group with mean of 8.1 days and highest in the azithromycin group with a mean of 8.7 days (p value 0.104). All the patients had fever with the most common associated feature being chills, followed by myalgia. It was seen maximum in doxycycline group (68.2%). Cough was the third common symptom, more commonly seen in azithromycin group (48.4%). The other clinical features were headache, vomiting abdominal pain and seizures. Seizures were more common in the combined group of azithromycin and doxycycline group.

Diabetes mellitus was seen in all the three groups, seen in 12.7% in doxycycline group, 10.4% in the combined group of azithromycin and doxycycline group. Scrub

IgM was positive in 82.8% in the azithromycin group, 77.1% in the combined group and 73% in the doxycycline group. (Table 6)

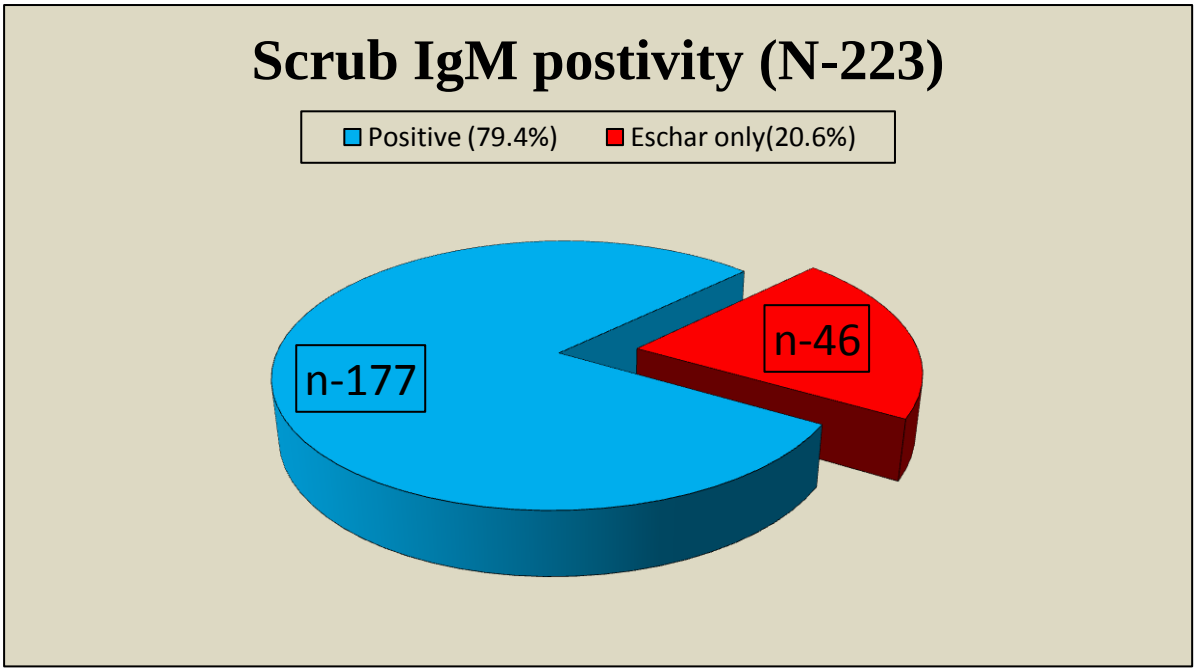
- **Laboratory characteristics in patients with Scrub Typhus**

Table 7:Laboratory features of patients with Scrub Typhus (N=223)	
Variable(N=223)	**Frequency, mean± SD**
Hemoglobin gm/dl	11.8 ± 2.34
Total leukocyte count, cells/mm^3	11,957 ±5450.9
Neutrophils%	74.74 ±11.3
Lymphocytes%	17.95 ±10.9
Platelets cells/mm^3	84,904 ±75489.4
Creatinine mg/dl	1.5 ±1.1
Urea mg/dl	61.09 ±50.6
Total bilirubin mg/dl	1.94 ±2.2
Direct Bilirubin mg/dl	1.60 ±2.1
Total Protein gm/dl	6.3 ±0.9
Albumin gm/dl	2.75 ±0.6
SGOT* IU/L	139.7 ±151.1
SGPT* IU/L	72 ±61.8
ALP* IU/L	187 ±131.6
Bicarbonate mmol/dl	19.05 ±4.8
*SGOT- Serum glutamic oxaloacetic transaminase, SGPT- Serum glutamic pyruvic transaminase, ALP- Alkaline phosphatase	

The mean hemoglobin was 11.8mg/dl±2.34 with the mean leukocyte counts of 11,957±5450.9 cells/mm^3, with neutrophils of 74.74±11.33 cells/mm^3. Thrombocytopenia was a common feature of scrub typhus with mean platelet counts of 84,904± 75,489 cells/mm^3 (Table 7).

The mean creatinine was 1.5±1.14mg/dl, mean urea of 61.09±50.59 mg/dl, liver function tests were derranged with mean total bilirubin of 1.94±2.18 mg/dl, mean albumin of 2.75±0.625 mg/dl, mean SGOT and SGPT was 139.7±151.12 mg/dl and 72±61.86 mg/dl respectively, the mean alkaline phosphatase was 187±131.61 mg/dl. Scrub IgM Elisa was positive in 79.4% of the patients with scrub typhus (Figure 22).

Figure 22: Scrub Typhus IgM ELISA positive in patients with Scrub Typhus



- **Comparison of laboratory parameters in the three antibiotic groups**

Table 8:Laboratory variables between the three antibiotic groups (N=223)

Variable	Doxy n=63, mean (SD)	Azithromycin n=64, mean (SD)	Doxycycline+Azithromycin, n=96, mean (SD)	*P* value
Haemoglobin gm/dl	12.3(1.93)	11.15(2.4)	11.8(2.4)	0.024
TLC* cells/mm^3	10972(5906)	11386(4581)	12973(5559)	0.023
Neutrophils %	71.73(12.3)	75.84(13.06)	75.95(10.5)	0.073
Lymphocytes%	19.97(10.7)	17.91(12.6)	16.67(9.6)	0.147
Platelet count cells/mm^3	99501(94815)	79093(65551)	79197(66143)	0.367
Creatinine mg/dL	1.16(0.8)	1.50(1.1)	1.72(1.2)	0.024
Urea mg/dL	43.50(32.6)	61.12(40.3)	71.28(61.6)	0.004
Total Bilirubin mg/dL	1.25(1.7)	2.18(2.6)	2.30(2)	<0.001
Direct Bilirubin mg/d	0.86(1.5)	2(2.9)	1.82(1.7)	<0.001
Protein gm/dL	6.73(0.8)	6.12(1.1)	6.1(0.82)	0.996
Albumin gm/dL	3.08(0.5)	2.63(0.5)	2.62(0.64)	<0.001
SGPT* IU/L	104.4(72.5)	132.6(89.1)	167.5(207.4)	0.009
SGOT* IU/L	73.4(66.9)	73.8(60.1)	70.2(60.1)	0.745
ALP* IU/L	132.8(84.5)	217.3(163.7)	201.3(122.9)	<0.001
Bicarbonate mmol/L	20.6(4.1)	20(4.5)	18.08(5.1)	0.006

Table 8-Laboratory variable between the three antibiotic groups (continued)				
Variable	**Doxy n=63, mean (SD)**	**Azithromycin n=64, mean (SD)**	**Doxycycline+Azithromycin, n=96, mean (SD)**	***P* value**
Scrub IgM positive n=177, (%)	46(73%)	57(82.8%)	74(77.1%)	0.063
SOFA*	3.60(2.3)	6.67(4.7)	8.26(5.2)	<0.001
*TLC-total leukocyte count, SGOT- Serum glutamic oxaloacetic transaminase, SGPT- Serum glutamic pyruvic transaminase, ALP- Alkaline phosphatase, SOFA- Sequential Organ Failure Assessment				

The comparison of the three groups showed that the hemoglobin level was higher in the doxycycline group with a mean of 12.37±1.93 mg/dl, the total counts was higher in the combined group of doxycycline and azithromycin with a mean of 12,973 cells/mm^3 (p value 0.024) . Thrombocytopenia was seen in all the three groups of the study with maximum thrombocytopenia seen in the azithromycin group (mean of 79,093cells/mm^3). Renal dysfunction was seen in two groups namely azithromycin and the combined group of doxycycline and azithromycin groups, with the maximum dysfunction seen in the combined group with creatinine of 1.72mg/dl. Hepatic dysfunction was highest in the combined group of azithromycin and doxycycline with total bilirubin of 2.30mg/dl, albumin of 2.62mg/dl, SGOT of 167.5U/L, SGPT of 70.2U/L, and alkaline phosphatase of 201.3IU/L.

- **Organ dysfunction in patients with scrub typhus**(4)

Table 9: Organ dysfunction in patients with scrub typhus (N-223)	
Organ involved	**Frequency n(%)**
Central nervous system involvement (altered sensorium)	64 (28.7%)
Cardiovascular dysfunction (BP<80mmhg)	24 (10.8%)
Respiratory dysfunction (SPO2<90)	112 (50%)
Hematological dysfunction	
Anemia (Hb<12gm/dl)	116 (52%)
Thrombocytopenia(<1lakh/mm^3)	150 (67.2%)
Hepatic dysfunction	
Elevated bilirubin (>2mg/dl)	44(19.7%)
SGOT (>3time 120IU/L)	92 (41.2%)
SGPT (>3 time 105IU/L)	38 (17%)
ALP (>140IU/L)	129 (57.8%)
Albumin (<3.5mg/dl)	198 (88.8%)
Renal dysfunction (Creatinine >2mg/dl)	46 (20.6%)
*TLC-total leukocyte count, SGOT- Serum glutamic oxaloacetic transaminase, SGPT- Serum glutamic pyruvic transaminase, ALP- Alkaline phosphatase	

- **Organ dysfunction in antibiotic groups**

Table 10: Organ dysfunction in antibiotic groups			
Organ dysfunction	**Doxycycline n=63, n (%)**	**Azithromycin n=64, n (%)**	**Doxy + azithro n=96, n (%)**
Central nervous system dysfunction (altered sensorium)	3(4.8%)	18 (28.1)	43 (44.8%)
Hemodynamic dysfunction (BP<80mmhg)	3(4.7%)	6(9.3%)	15(15.6%)
Respiratory dysfunction (SPO2<90)	36(57.1%)	26(40.6%)	50(52.1%)
Hematological dysfunction			
Anemia (Hb<12gm/dl)	29(46%)	38(59.4%)	49(51%)
Thrombocytopenia(<1lakh/mm^3)	42(66.6%)	45(70.3%)	63(65.6%)
Hepatic dysfunction			
Elevated bilirubin (>2mg/dl)	5(7.9%)	11(17.1%)	28(29.1%)
SGOT (>3 times 120IU/L)	22(34.9%)	28(43.7%)	42(43.7%)
SGPT (>3 times, 105IU/L)	12(19%)	11(17%)	15(15.6%)
ALP(>125IU/L)	22(34.4%)	42(65.6%)	65(67.7%)
Albumin (<3.5mg/dl)	46(73.8%)	61(95.3%)	91(94.8%)
Renal dysfunction (Creat>2mg/dl)	4(6.3%)	14(21.8%)	28(29.1%)
*TLC-total leukocyte count, SGOT- Serum glutamic oxaloacetic transaminase, SGPT- Serum glutamic pyruvic transaminase, ALP- Alkaline phosphatase.			

Table 11:Number of organs involved in patients with scrub typhus (N=223)	
Number of organs involved (n)	**Frequency, n (%)**
0	12 (5.4)
1	40 (17.9)
2	82 (36.8)
3	51 (22.9)
4	29 (13)
5	8 (3.6)
6	1 (0.4)

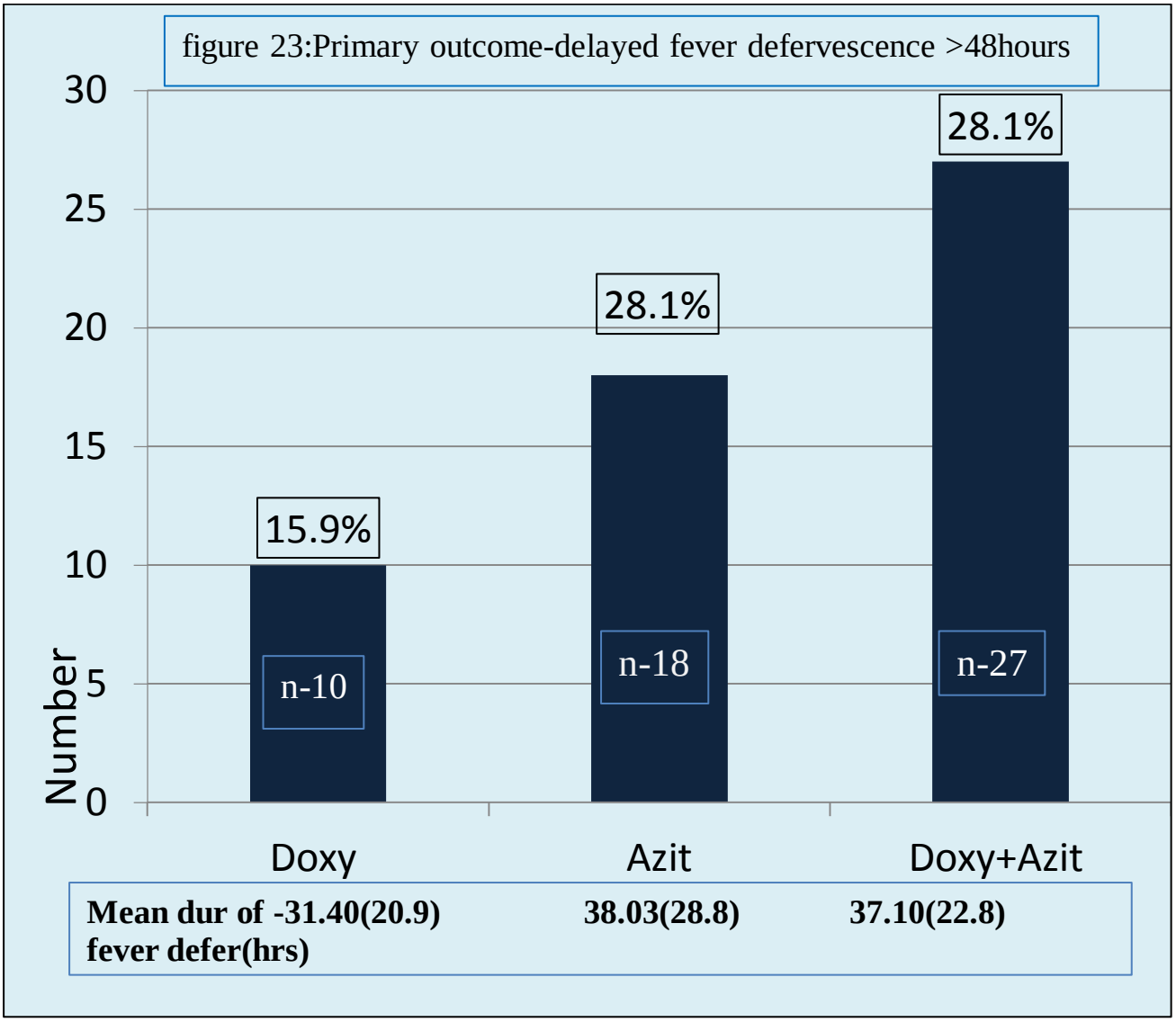
The most common organ dysfunction in our cohort was hematological dysfunction followed by respiratory and hepatic dysfunction. The central nervous system and renal dysfunction were more severe in the combined group; respiratory dysfunction was more common in the doxycycline group. Two organ systems were commonly involved (36.8%).

OUTCOMES

Primary Outcome in patients with scrub typhus

Table 12: Primary outcome, Incidence of delayed defervescence in patients with scrub typhus (N=223)				
Variable	**Doxycycline** **n- 63**	**Azithromycin** **n- 64**	**Doxycycline+ Azithromycin** **n-96**	
Mean duration of fever defervescence, hours (SD)	31.40(20.9)	38.03(28.8)	37.10(22.8)	*P* value- 0.257
Delayed fever defervescence (>48hrs), mean (%)	10 (15.9)	18 (28.1)	27 (28.1)	Total=55 (24.66%)
Fever defervescence (<48 hrs), mean (%)	53 (84.1)	46 (71.9)	69 (71.9)	Total=168 (75.64%)

Figure 23: Primary outcome- delayed defervescence in patients with scrub typhus.



The primary outcome was incidence of delayed defervescence which was defined as the duration of fever defervescence of more than 48 hours after the initiation of antibiotics in patients presenting with scrub typhus. The mean duration of fever defervescence in the three groups, doxycycline, Azithromycin and the combined group of doxycycline and Azithromycin group was 31.40 hrs, 38.04 hrs, 37.10 hrs respectively (p value 0.257). The incidence of delayed defervescence in patients with

scrub typhus presenting to the hospital was 24.66% (n=55). The number of patients with delayed defervescence was lowest in doxycycline group 15.9% (n=10).

(Table 12)

Secondary outcomes

Subgroup analysis was done to the patients to look at the secondary outcomes which showed the following.

- **Factors associated with delayed defervescence (Univariate analysis).**

Table 13: Comparison between early and delayed defervescence group in all the patients with Scrub typhus (n=223)

Variable (n=223)	**Delayed defervescence** >48 hours (n=55)	**Fever defervescence** < 48hours (n=168)	***P* value**
Sex	**n (%)**	**n (%)**	
Male	24(43.6%)	78(43.6%)	0.718
Female	31(56.3%)	90(53.6%)	
Clinical features			
Duration of illness, days (SD)	7.98(3.6)	8.45(4.2)	0.922
Breathlessness (%)	52(94.4%)	145(86.3%)	0.145
Diabetes mellitus (%)	5(9.09%)	18(10.7%)	0.731
Laboratory parameters	**Mean (SD)**	**Mean(SD)**	
Haemoglobin gm/dl	11.9(2.3)	11.7(2.3)	0.575
TLC* /mm^3	11670(5741)	12051(5366)	0.398
Platelets cells/mm^3	69729(58071)	89872(9801)	0.102
Creatinine mg/dl	1.3(0.9)	1.5(1.2)	0.446

Table 13- Comparison between early and delayed defervescence group in all the patients with scrub typhus (continued)

Variable (n=223)	Delayed defervescence >48 hours (n=55)	Fever defervescence < 48hours (n=168)	*P* value
Total bilirubin mg/dl	1.9(2.3)	1.9(2.1)	0.698
Albumin gm/dl	2.8(0.5)	2.7(0.6)	0.549
SGOT * IU/L	141.7(91.5)	139(166.3)	0.134
SGPT* IU/L	68.4(37.8)	73.3(67.9)	0.514
ALP* IU/L	185.4(128.5)	187.5(132.9)	0.897
Bicarbonate mmol/dl	19.6(4.1)	18.8(5)	0.383
SOFA score	7.1(4.9)	6.28(4.8)	0.215

*****TLC-total leukocyte count, SGOT- Serum glutamic oxaloacetic transaminase, SGPT- Serum glutamic pyruvic transaminase**,** ALP- Alkaline phosphatase, SOFA- Sequential Organ Failure Assessment

- **Organ dysfunction versus delayed defervescence (Univariate analysis)**

Table 14: Comparison of organ dysfunction versus defervescence in patients with Scrub typhus (Univariate analysis, N=223)			
Variable **(n=223)**	**Delayed defervescence** **>48 hours** **(n=55), Mean(SD)**	**Fever defervescence** **< 48hours** **(n=168), Mean (SD)**	***P* value**
Duration of hypotension, day	0.74(1.8)	0.41(0.9)	0.45
Duration of inotropes, days	0.69(1.8)	0.36(0.9)	0.51
Duration of oxygen, days	3(3.3)	1.6(2.4)	0.01
Duration of NIV, days	0.4(1.1)	0.2(0.8)	0.36
Duration of IV, days	1.7(3.4)	0.9(2.2)	0.19
Duration of ventilator, days	2.1(3.5)	1.2(2.3)	0.07
Duration of altered sensorium, days	1.7(3.3)	1(2.1)	0.56

- **Logistic regression**

Table 15: Logistic regression of significant variables	
Variable (n=223)	**Significance- *p* Value**
Duration of fever	0.457
Breathlessness	0.099
SOFA score	0.272
Platelet count	0.085
Duration of oxygen (days)	0.001
Duration of ventilator (days)	0.020

- **Comparison between the number of organs involved and delayed defervescence**

Table 16: Comparison between the number of organs involved and delayed defervescence			
Organs involved	**Early defervescence, n %)**	**Delayed defervescence, n (%)**	**Odds ratio**-14.58, **95% C.I**.- (6.71-32.86) **P value**-<0.001.
>2 organs	43 (25.6)	46 (83.6)	
≤ 2 organs	125 (74.4)	9 (16.3)	

Univariate analysis of baseline characteristics showed that the respiratory system involvement in the form of duration of oxygen required and duration of ventilator required were statistically significant with respect to the primary outcome. Logistic regression done for the factors that were trending towards significance which did not show statistical significance showed that respiratory system involvement in the form of increasing duration of oxygen and duration of ventilator days had increasing incidence of delayed defervescence (*p* value 0.001 & 0.020). It was also seen that the incidence of delayed defervescence increased when more than 2 organ systems were involved (83.3%, p value <0.001) with odds ratio of 14.58 (6.71-32.86).

- **Duration of organ recovery**

Table 17: Comparison between antibiotic groups for organ recovery (N=223)

Variable	Doxycycline (n=63), mean(SD)	Azithromycin (n=64), mean(SD)	Doxycycline+Azithromycin (n=96), mean(SD)	*P* value
SOFA *	3.60(2.3)	6.67(4.6)	8.26(5.2)	*<0.001*
Duration hospital stay days	4.16(2.7)	6.97(3.6)	6.51(3.6)	*<0.001*
Duration of hypotension days	0.05(0.21)	0.50(1.09)	0.79(1.6)	*<0.001*
Duration of Inotrope days	0	0.47(1.05)	0.72(1.6)	*<0.001*
Duration of Oxygen days	0.38(1.09)	1.75(2.5)	3.10(3.09)	*<0.001*
Duration of NIV* days	0.16(0.8)	0.30(0.8)	0.45(1.05)	*<0.001*
Duration of IV* days	0.06(0.5)	1.00(2.3)	1.88(3.2)	*<0.001*

Table 17-Comparison between antibiotic groups for organ recovery (continued)

Variable	Doxycycline (n=63), mean(SD)	Azithromycin (n=64), mean(SD)	Doxycycline+Azithromycin (n=96), mean(SD)	*P* value
Duration of Low altered sensorium* days	0.10(0.5)	1.11(2.3)	2.06(3.09)	*<0.001*
Duration of creatinine >2mg/dl, days	0.25(0.9)	0.86(2)	1.15(2.1)	*0.003*
Duration of bilirubin >2mg/dl, days	0.44(1.3)	1.56(2.6)	1.74(2.3)	*0.001*

*SOFA- Sequential Organ Failure Assessment, NIV- non invasive ventilation, IV- invasive ventilation, GCS- Glasgow coma scale

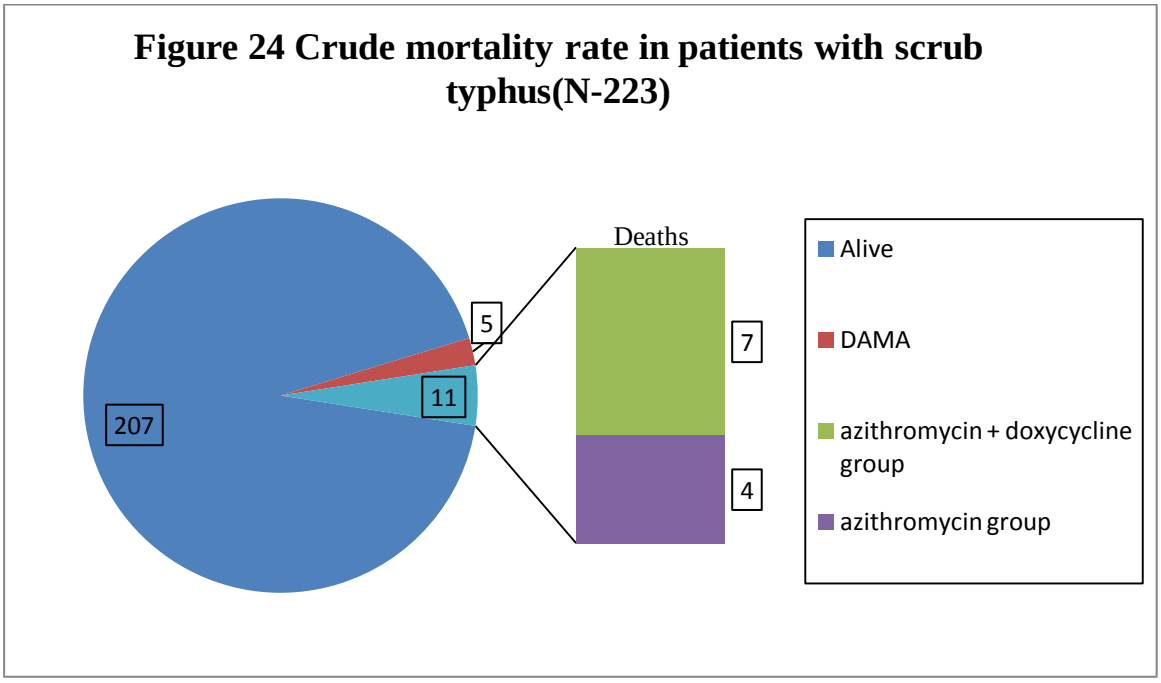
The combined group of Doxycycline and Azithromycin had patients with severe illness as evidenced by SOFA (sequential organ failure assessment) score of 8.26, Doxy group and Azithromycin groups had SOFA score were 3.60 and 6.67 respectively. The total hospital stay days was longer in Azithromycin group with mean duration of 6.97 days, doxy and Azithromycin group were 4.16 and 6.51 days respectively (p <0.001). The total days of hypotension was longest in the 0.79 days (p value <0.001) combined group of Doxycycline and Azithromycin group, the total inotrope days were higher in the combined group of Doxycycline and Azithromycin group with a mean of 0.72days (p value <0.001).

The oxygen requirement was maximum in the combined doxycycline and Azithromycin group with a mean of 3.10days (p value <0.001). The requirement of mechanical ventilation with non invasive ventilation (NIV) and invasive ventilation (IV) was highest in the combined antibiotic group of doxycycline and Azithromycin group with a mean duration of 0.45 days and 1.88 days respectively (p< 0.001).

The sensorium took longer to recover in the combined group of doxycycline and Azithromycin with a mean of 2.07days (p <0.001) of total reduced GCS (Glasgow coma scale) days.

The renal failure took longer to recover in the combined group of Azithromycin and Doxycycline group with mean of 1.15days (p 0.003).The total days of elevated bilirubin of more than 2mg/dl was higher in the combined group of Azithromycin and Doxycycline group with a mean duration of 1.74days (p 0.001) (Table 19).

Figure 24: Crude mortality rate in patients with scrub typhus



There were a total of 11 deaths in the cohort with a crude mortality rate of 4.9%, with 7 in the combined group and 4 in the azithromycin only group, 5 were discharged against medical advice (DAMA).

Results in critically ill patients

- **Baseline characteristics in critically ill patients (ICU)**

Table 18:Baseline characteristics of critically ill patients (N=56)	
Variable (n=56)	**Frequency (mean ± SD)**
Duration of fever, days	9.46±8.98
SOFA Score	12.91±4.33
Pulse /min	118.5± 23.11
Systolic Blood Pressure mm of Hg	102.32 ±23.19
Diastolic Blood Pressure mm of Hg	63.04 ±15.00
Respiratory Rate /min	38.48 ±10.35
Laboratory parameters	**Frequency (mean ± SD)**
Haemoglobin gm/dl	11.42 ±2.84
TLC* cell/mm^3	13001 ±5668.60
Neutrophils (%)	77.25 ±12.53
Lymphocytes (%)	17.02 ± 11.56
Platelets cell/mm^3	55,535 ± 49335.57
Creatinine mg/dL	2.07 ± 1.42
Total bilirubin mg/dL	2.74 ± 2.47
Direct bilirubin mg/dL	2.205 ±2.21
Total Protein g/dL	6.664 ±6.09
Albumin g/dL	2.50 ±0.716
SGOT* IU/L	179.48± 134.28
SGPT*IU/L	63.82 ±43.43

Table 18- Baseline characteristics of critically ill patients (continued)	
Variable (n=56)	**Frequency (mean ± SD)**
ALP* IU/L	234.68 ±129.04
Bicarbonate mmol/L	15.82 ±4.63
*TLC-total leukocyte count**,** SGOT- Serum glutamic oxaloacetic transaminase, SGPT- Serum glutamic pyruvic transaminase, ALP- Alkaline phosphatase**.**	

There were 56 subjects requiring critical care. The mean duration of fever at presentation was 9.46±8.98 days. The mean pulse rate was 118.5±23.11 bpm, mean systolic blood pressure was 102.32 mm of hg (SD 23), mean diastolic blood pressure was 63.04 mm of hg (SD15), mean respiratory rate was 38.48 per minute (SD 10.35) (Table 20).

- **Laboratory parameters in critically ill patients**

Table 19:Comparison of laboratory parameters in the three antibiotic groups in critically ill subjects (N=56)

Variable (n=56)	Doxycycline (n=2), mean(SD)	Azithromycin (n=15) , mean(SD)	Doxycycline +Azithromycin (n=39) , mean(SD)	*P* value
SOFA score	10.50(7.7)	12.80(5)	13.08(3.9)	0.852
Haemoglobin g/dl	9.9(0.9)	11.4(1.9)	11.5(3.1)	0.388
TLC* cell/mm^3	17,500(2404)	10109(4889)	13883(5690)	0.029
Neutrophils %	75.5(14.8)	80.3(13.3)	76.2(12.3)	0.324
Platelet count cell/mm^3	38,500(20506)	66,000(44357)	52,384(52136)	0.218
Creatinine mg/dl	2.13(1.8)	2.20(1.6)	2.02(1.3)	0.920
Total Bilirubin mg/dl	1.75(1.5)	3.34(3.8)	2.56(1.8)	0.907
Albumin g/dl	2.30(0.1)	2.31(0.4)	2.58(0.8)	0.320
SGOT* IU/L	110.00(38.2)	167.4(107.8)	187.69(146.2)	0.772
SGPT * IU/L	20.00(5.6)	73.20(39.4)	62.46(44.9)	0.053
ALP* IU/L	216.00(53.7)	229.80(109.7)	237.51(139.8)	0.996
Bicarbonate mmol/L	14.00(8.5)	15.13(3.2)	16.17(4.9)	0.622

*****TLC-total leukocyte count**,** SGOT- Serum glutamic oxaloacetic transaminase, SGPT- Serum glutamic pyruvic transaminase, ALP- Alkaline phosphatase**.**

The mean hemoglobin was 11.42 $\pm$ 2.84 mg/dl, mean total count 13001±5668.6 cells/mm^3, and mean platelet count was 55,535$\pm$49335.5 cells/mm^3.The mean creatinine value in critically ill patients was 2.07±1.42 mg/dl, mean total bilirubin was 2.74±2.47mg/dl, mean albumin was 2.50±0.716mg/dl, there was evidence of hepatitis with elevated liver enzymes, with mean SGOT and SGPT of 179.48±134.28U/L and

63.82±43.43U/L respectively and the mean alkaline phosphatase level was 234.68±129.04U/L. The patients were generally severely ill with higher mean SOFA score levels were 12.91±4.33.

The hemoglobin was least in the doxycycline group with a mean value of 9.90gm/dl (p value 0.388), total counts was maximum in the doxycycline group with a mean value of 17,500cells/mm^3 (p value 0.029), thrombocytopenia was seen in all the three groups with lowest platelet levels in the doxycycline group with mean value of 38,500cells/mm^3 (p value of 0.218). Renal dysfunction was maximum in the azithromycin group with mean creatinine of 2.20mg/dl (p value 0.920); hepatic dysfunction was maximum in the azithromycin group with a mean total bilirubin of 3.34 mg/dl (p value 0.907), transaminitis was severe in the combined group of azithromycin and doxycycline group with a mean SGOT and SGPT of 187.69 U/L (p value 0.772) and 62.46 U/L (p value 0.053) respectively. The general severity of the disease was more in the combined group of azithromycin and doxycycline with a mean SOFA of 13.08 (p value of 0.852).

Table 20: Organ dysfunction in critically ill patients		
Organ dysfunction	**Monotherapy (Doxy or azithromycin, N=17, n (%)**	**Dual therapy (Doxy + azithro) N=39, n (%)**
Central nervous system dysfunction(altered sensorium)	14 (82.3%)	29(74.3%)
Hemodynamic dysfunction (BP≤80mmhg)	2(11.7%)	10(25.6%)
Respiratory dysfunction (SPO<90%)	10(58.8%)	15(38.4%)
Hematological dysfunction		
Anemia (Hb<12gm/dl)	10(58.8%)	31(79.4%)
Thrombocytopenia(<1lakh/mm^3)	17(100%)	32(82.05%)
Hepatic dysfunction		
Elevated bilirubin (≥2mg/dl)	9(52.9%)	12(30.7%)
SGOT (>3times 120IU/L)	12(70.6%)	10(25.6%)
SGPT (>3times 105IU/L)	5(29.4%)	8(20.5%)
Alkaline phosphatase (>125IU/L)	12(70.6%)	27(69.2%)
Albumin (≤3.5mg/dl)	15(88.23%)	33(84.6%)
Renal dysfunction (Creat≥2mg/dl)	9(52.9%)	22 (56.4%)

The organ system involvement was similar in both the monotherapy and combination therapy arms. Central nervous system, hematological and hepatic involvement was more seen in the monotherapy arm, renal dysfunction was seen in the combination group.

Outcomes in critically ill subjects

Primary outcome in critically ill patients

Table 21:Incidence of delayed defervescence in critically ill patients with Scrub Typhus(n=223)

Variable (N= 56)	Doxycycline (n=2)	Azithromycin (n=15)	Doxycycline+Azithromycin (n=39)	
Duration of fever defervescence, hours. mean (SD)	9(11.3)	37.20(36)	37.15(23.9)	*P* value-0.248
Delayed defervescence >48hrs, n (%)	0	4 (26.7)	12 (31.6)	16(28.6%)

The primary outcome, incidence of delayed defervescence in critically ill patients was 28.6% (n=16) (p value 0.248). The mean duration of fever defervescence in doxycycline group was the lowest with a mean of 9 hours, defervescence in Azithromycin and the combined doxycycline and Azithromycin group was 37.20hrs and 36.15 hrs respectively (p value 0.248). The incidence of delayed defervescence was highest in the combined group of doxycycline and Azithromycin, 21.40% (n=12). (Table 21)

Secondary outcomes in critically ill patients

- **Comparison between early and delayed defervescence groups in critically ill patients with scrub typhus(Univariate analysis)**

Table 22: Comparison between early and delayed defervescence groups in critically ill patients with scrub typhus (N=56)

Variable (n-56)	Fever defervescence <48hrs n=40, mean(SD)	Delayed defervescence >48 hrs n=16, mean(SD)	*P* Value
Duration of illness, days	9.98(10.5)	8.19(2.7)	0.822
SOFA* score	12.55(4.4)	13.81(4.1)	0.30
Pulse /min	117.70(23.2)	120.69(25.7)	0.84
SBP* mmHg	99.75(20.8)	108.75(23.3)	0.95
RR* /min	38.78(11.8)	37.75(8)	0.87
Haemoglobin gm%	11.43(2.5)	11.38(2.4)	0.90
TLC* cells/mm^3	13037(5963)	12913(5140)	0.73
Neutrophils %	77.68(11.4)	76.19(14.2)	0.95
Platelets cells/ mm^3	53625(42126)	60312(54763)	0.69
Creatinine mg/dl	2.14(1.4)	1.38(1.3)	0.47
Total bilirubin mg/dl	2.66(2.1)	2.94(3.4)	0.72
Albumin gm/dl	2.48(0.8)	2.55(0.39)	0.19
SGOT* IU/L	178.88(105.4)	181.00(80.1)	0.67
SGPT* IU/L	62.28(57.7)	67.69(39.6)	0.44
ALP* IU/L	229.73(103.9)	247.06(161.2)	0.80
Bicarbonate mmol/L	15.34(4.5)	17.01(4.55)	0.26

The mean pulse rate was highest in the delayed defervescence group with a mean of 120.69 /min when compared to the early defervescence group which was 117.70 /min. The blood pressure at presentation was highest in the delayed defervescence group with mean systolic blood pressure of 108.75mmhg (Table 22). The duration of initial presentation of fever was least in the delayed defervescence group with a mean of 8.19 days. Renal dysfunction was the highest in the early defervescence group with a mean creatinine of 2.14 mg/dl (p value 0.47). The alkaline phosphatase and SGOT values were higher in the delayed defervescence group 247.06 U/L (p value 0.67) and 181 U/L (p value 0.44) respectively.

- **Duration of organ recovery in critically ill patients with scrub typhus**

Table 23:Comparison between the antibiotic groups for organ recovery in critically ill patients (N=56)

Variable (n-56)	Monotherapy (Doxy or azithromycin) n=17, mean(SD)	Doxycycline+Azithromycin n=39 , mean(SD)	*P* value
SOFA* score	12.52(5.1)	13.08(3.9)	0.077
Duration of ICU* days	4.76(3.3)	5.36(2.9)	0.471
Total duration of hospital admission day	8.23(3.9)	9.10(3.9)	0.745
Total duration of Hypotension, days	1.41(1.4)	1.85(2.2)	0.163
Total duration of Inotrope, days	1.29(1.3)	1.62(1.6)	0.249
Total duration of oxygen, days	5(2.6)	5.54(2.9)	0.111
Total duration of low p/f* days	4.7(2.8)	5.54(2.9)	0.443
Total duration of altered sensorium, day	3.76(3.2)	4.77(3.2)	0.794
Duration of NIV*, days	1(1.9)	0.67(1.3)	0.085
Duration of IV*, days	3.52(3.3)	4.64(3.6)	0.848

*SOFA- Sequential Organ Failure Assessment, ICU-intensive care unit, p/f –PaO2/FiO2, GCS- Glasgow coma scale, NIV- non invasive ventilation, IV- invasive ventilation.

The combined group of doxycycline and azithromycin group had patients with severe illness with a mean SOFA score of 13.08, with doxycycline and azithromycin groups having SOFA scores of 10.50 and 12.80 respectively (p value 0.852). The combined group of doxycycline and azithromycin group had shorter mean ICU days of 5.36 days

compared to doxycycline group with mean of 7 days (p value 0.471). The total hypotension days was longer in combined group of doxycycline and azithromycin group with a mean of 1.85 days compared to azithromycin and doxycycline with mean of 1.47days (p value 0.602).

The total inotrope requirement was higher in the combined group of doxycycline and azithromycin group with mean of 1.62days, compared to azithromycin with mean of 1.47 days (p value 0.276).

The total oxygen requirement was lower in azithromycin group with a mean of 4.93days, compared to doxycycline and the combined group of doxycycline and azithromycin with a mean of 5.50 days and 5.54 days respectively (p value 0.872). The non invasive ventilation (NIV) requirement was longer in doxycycline group with a mean of 3.50 days, compared to azithromycin and the combined group of azithromycin and doxycycline group , mean of 0.67 days and 0.67 days (p value 0.556). The total days of invasive ventilation (IV) was longer in the combined group of doxycycline and azithromycin with a mean of 4.64 days, compared to doxycycline group with mean of 2 days and azithromycin group with mean of 3.73 days (p value 0.486). The sensorium recovery was faster in azithromycin group with a mean of 3.53 days (p value 0.439). (Table 23)

DISCUSSION

Scrub typhus is a common cause of acute febrile illness in the tsutsugamushi triangle, is caused by a rickettsial organism *Orientia tsutsugamushi*. In this cohort 241 patients were screened and 223 satisfied the inclusion criteria, the incidence of delayed defervescence was highest in this study (24.66%) compared to previous studies.

The mean age of 47.6 ± 15.1 years was comparable to studies from South India, mean age (40-46 years)(4,8,41,46,84). Females were more commonly affected than men, which was similar to studies done by Kim et al and Griffith et al (7,7,41). However male predominance was seen in studies from South India(4,46). There is a change in trend towards female predominance which could have been because of increasing number of women working in the fields. Scrub typhus usually occurs in the occupations which have an increased exposure to the scrub vegetation and around houses. Studies from our institution had shown housewives to be the predominant occupation which was also seen in our cohort, the other professions being farmers , manual labourer.(46)

The mean duration of illness depends on the time taken by the subjects to seek medical attention and also the incubation period, it was 8.33 days in our study which was similar to studies done in the same geographic areas of south India (mean 8-8.5days)(4,41).

The eschar which is a characteristic feature of scrub typhus indicates initial site of inoculation by the chigger through which the organism enters the lymphatic and systemic dissemination, thus forming an important diagnostic feature(85). The eschar detection rate is very variable ranging from 37.6-55% (4,4,41,46,84). The detection

rate of 72.2% in this study was one of the highest in the country. This may have been because of increasing awareness of the disease and vigilance in searching for this vital diagnostic feature. The detection rates are very high in the orient (Japan and Korea) which may be due to the racial differences in the skin colour aiding in easy identification(86,87).

Scrub typhus commonly causes multiorgan dysfunction leading to high rates of mortality if not treated. The respiratory system is predominantly involved with very high incidence(68.5%-73.5%) (4,41). The pathogenesis is most probably because of interstitial pneumonia with or without vasculitis(38). In our cohort, though breathlessness (88.3%) was the common presenting symptom, respiratory dysfunction requiring oxygen was seen in about half of the subjects (50%). This emphasizes the fact that patients presenting with acute undifferentiated febrile illness with respiratory dysfunction increases the probability of scrub typhus in this geographic area. CNS dysfunction is seen in the form of meningoencephalitis, encephalomyelitis or focal neurological deficits, with altered sensorium being the most common presenting feature (22-32%) (46). In our cohort critically ill subjects had higher involvement of the CNS.

Thrombocytopenia is a characteristic laboratory feature and can be seen in very high proportion of subjects. It is caused secondary to localized and generalized vasculitis. (4, 41).In our study hematological system was commonly involved with a very high percentage of subjects having thrombocytopenia, with higher proportion in the critically ill subjects. Majority of our patients had leukocytosis. In a tropical country like India, dengue, an arboviral illness has similar presentation as scrub typhus and is

found in the same geographical areas. This often causes difficulty in diagnoses in the absence of protean manifestations of scrub typhus. Presence of leukocytosis with multiorgan dysfunction may help in considering scrub typhus as a diagnostic possibility.

Hepatic dysfunction in the form of transaminitis is a very consistent finding (60-90%) in the majority of the subjects in several of the studies. (46, 47) The patients in the critically ill group had more liver dysfunction, indicating liver is a prime target in severe scrub typhus. We noted that hepatocellular pattern of injury in the form of enzyme elevation was the predominant type of liver injury.

Renal dysfunction ranges from acute renal failure requiring dialysis to microscopic proteinuria and pyuria. It is seen in a variable proportion (18-30%) of subjects with increased involvement in critically ill subjects (68.3%) (4,21,41,42). In our study we noticed that renal dysfunction was common (20.6%) and was comparable to earlier studies with an obviously higher percentage in the critically ill patients.

Hypoalbuminemia is a common finding in scrub typhus, which is caused due to cytokine and cytotoxic damage leading to vascular leakage. Lower albumin has been used as a predictor of mortality in scrub typhus(7)(48). In this cohort also lower albumin levels were seen in majority of subjects, indicating severity of illness at presentation and diffuse systemic involvement of the disease.

Rapid fever defervescence after initial antibiotic therapy is considered a characteristic feature of scrub typhus with a delay in defervescence usually suggestive of an alternate diagnosis. The incidence of delayed defervescence was only about 10% in a study done on US servicemen during Vietnam War with a mean duration of fever

being 27.8hours, which was similar to study by Brown et al from Malaysia (88,89). However studies in the last two decades showed an increase in the incidence of delayed defervescence following antimicrobial therapy which ranged from 18.4%-21.5%(7,8). This delay in defervescence had been postulated to be due to infection with doxycycline resistant strains, more virulent strains or inadequate drug levels (7,8). A study from northern Thailand in 1996 had detected doxycycline resistant strains in the cell culture that could be due to be an increased selection of resistant strains in the mite chiggers(90). However in vivo resistance has never been proven. We hypothesized that the delay in defervescence is due to severity of illness and not due to pathogen factors.

Our incidence of delayed defervescence (24.66%) was the highest ever reported. It was seen that the odds of patients with multiorgan involvement (more than 2 organs) having delayed defervescence was 14.6 times compared to those with less than one organ involvement. Respiratory system involvement was found to the independent predictor of delayed defervescence. Thus in this cohort severity of organ dysfunction was the likely determinant of increased defervescence time. Choice of antimicrobial therapy did not seem to affect the defervescence time.

Most patients on monotherapy with doxycycline had single organ involvement and hence had lower rates of delayed defervescence as compared to dual therapy where majority had multiple organ involvement (15% v/s 28.1%).

The combined severity of illness was assessed using SOFA score which is a combined scoring system, the SOFA score has never been studied in scrub typhus till now. The combined antibiotic group had higher SOFA (8.26) compared to the monotherapy

groups. In the critically ill subjects also the mean SOFA score (13.81) was higher in the combined antibiotic group. This denotes that combined therapy was preferred in the more severely ill subjects by the treating physicians. This uneven distribution may have resulted due to the selection bias that could have occurred in this study design.

Scrub typhus affects multiple organs leading to dysfunction and failure and if left untreated mortality is seen in considerable proportion of subjects. This is the first prospective study that looked at the organ recovery following antimicrobial therapy. In this cohort the organ recovery appeared to be shorter in the doxycycline group compared to the other two antibiotic groups with respect to total hospital stay, inotrope requirement, oxygen requirement, ventilator requirement, CNS, renal dysfunction and hepatic dysfunction recovery. Thus the treatment with doxycycline as monotherapy was apparently better than the other two groups; however there could have been a selection bias with respect to severely ill subjects receiving combined therapy. Therefore further prospective randomized studies would be required to study the organ recovery with respect to different antibiotics.

CONCLUSIONS OF THE STUDY

- The incidence of delayed defervescence was 24.66%, with the incidence in doxycycline group being 15.9%, and in azithromycin and the combined groups (azithromycin and doxycycline) being 28.1% and 28.1% respectively. The incidence of delayed defervescence in critically ill subjects was 28.6%.
- Respiratory system dysfunction and the multiorgan involvement (more than two organs) independently predicted delayed defervescence.
- Severity of organ dysfunction seems to determine the defervescence time rather than the choice of the antimicrobial therapy as postulated before.
- Further prospective randomized studies are required with regard to this and also to develop newer antibiotics to combat this curable rickettsial infection.

LIMITATIONS OF THE STUDY

1. This was an observational cohort study with no randomization of the antibiotic regimens used, leading to uneven distribution in the three groups.
2. The follow up period was set as 2 weeks, new symptoms recruited after discharge could have been missed.
3. Scrub IgM ELISA and or presence of eschar were used as a diagnostic criterion. However the gold standard for serological diagnosis is indirect fluorescent antibody (IFA) which was not available in our Institute. But prior studies have validated IgM ELISA in our laboratory and pretest probability that a febrile illness with multiorgan involvement is scrub typhus is very high in our region.
4. This being a tertiary referral care hospital, the profile could not be representative of the exact profile in the community.
5. The pre hospital antibiotic administration to the subjects could not be precisely determined, which could have had a confounding effect on the result. This was minimized by doing a thorough clinical evaluation.
6. Adequacy of antibiotics by checking the antibiotic levels in the blood was not done, which could have determined the pharmacokinetics of the drugs used.

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ANNEXURES

1. **PATIENT INFORMATION SHEET**

2. **PATIENT CONSENT FORM**

3. **DATA ABSTRACTION SHEET**

4. **DATA SHEET**

ANNEXURE 1

PATIENT INFORMATION SHEET

Scrub typhus is an infective disease that is caused by an organism called Rickettsia and is spread by the bite of a mite while walking in grasslands or forest. It usually causes a short duration fever which recovers after taking a short course of antibiotics, sometimes it causes a very serious illness affecting the lungs, brain, and blood, liver and can cause death if not treated adequately. Hence starting treatment with antibiotics early is very important.

This study is aimed at studying the pattern of illness with scrub typhus. The time taken for the disease to recover after starting the antibiotics will be studied. The effects of various antibiotics used in scrub typhus and their effects will be studied. This will help us compare the different antibiotics used and chose the best one in the treatment of this disease.

By participating in the study you will not be made to incur any added expenses apart from the routine initial investigations. There will be no added risk or discomfort or discomfort of any kind to you by participating in this study. Any personal information that is collected as a part of this study will be maintained strictly confidential. Participation is entirely voluntary and refusal to participate will not involve any loss of benefit or change that treatment that you receive.

ANNEXURE 2

PATIENT CONSENT FORM

I have been explained about the details of the study- *"Prospective observational study to determine the factors associated with outcome of scrub typhus (DDS Study).",* and I have also read the above information sheet and have understood the same. I hereby give my consent to participate in this study and my participation is voluntary.

Study number:

Subject's name: Hospital number:

Subject's signature/Thumb impression Investigators signature

Annexure 3- Data Abstraction sheet

Name: **Age:** **Sex**: M / F **DOA/1st visit**: …. /… /2012

Hosp No: **Occupation:** HW/Man labour/Student/Business/Farmer/…...

State: TN/AP/WB/……. **Unit:** OPD / Med 1 / Med 2 /Med 3/ Med 4

Contact Number: **Address:**

Village/ town:

Dur of fever	days	**Seizures**	Yes / No	No risk fac	Other-
Chills	Yes / No	**Abd pain**	Yes / No	DM	Yes/no
Gen myalgia	Yes / No	**Cough**	Yes / No	HTN	Yes/no
Head ache	Yes / No	**Breathless**	Yes / No	CVA	Yes/no
Vomiting	Yes / No	RED Urine out	Yes / No	CLD	Yes/no
Overt bleed	Yes/no Site:	**Altered sensor**	Yes / No	IHD	Yes/no
Smoker/alcoholic	/	**Pregnancy**		CKD	Yes/No

Pulse	/min		Crepitations	Yes/no
BP	/ mm Hg	MAP	splenomegaly	Yes/no
Resp rate	/min		Hepatomegaly	Yes/no
SPO2	%		Rash	Yes/no
Eschar	Yes/no Site: Groin/Axilla/			

Haemoglobin		Creatinine/urea	/	Bicarbonate	
TC		TB/DB	/		
Neutrophils	%	Protein/Albumin	/		
Lymphocytes	%	SGOT/SGPT	/		
Platelets		ALP			

SOFA(SF)	**0**	**1**	**2**	**3**	**4**	**(SF 1)**	**SF2**	**SF3**
P/F ratio	>400	≤400	≤300	≤200	≤100			
Platelets	>1.5L	1-1.5 L	50-1 L	20,000-50,000	<20000			
Bilirubin	<1.2	1.2-1.9	2-5.9	6-11.9	>12			
BP	No hypo	map<70	D<5	D>5/Ad<0.1	D>15/Adr>0.1			
GCS	15	13-14	10-12	6-9	<6			
Creat	<1.2	1.2-1.9	2-3.4	3.5-4.9	>5			

	Initiation	Fever defervescence

	Date	Time	Duration	Date	Time	Defer time
Doxy			days			
Azithro			days			
Doxy+ Azithro			days			
Others						

Antipyretics		Steroids	
Dose /dur		Drug	
Frequency	Prn/tid/bd	Dose /dur	
Indication		Frequency	

OP / IP	**If IP:** MICU / I / C / E / MTS4 / Isolation / Private					
Dur of adm	**MICU/MHDU**	days	**Ward**	days	**Total**	days
Supports	**NIV/IV**	**Oxygen**	**Inotropes**		**Dialysis(No)**	
Duration	**/ Total**		Ad/NA/D/V days			
Outcome at dis	Alive / Dead	**Stat at dis**	Afebrile / Fever persisting			

	Cardiovascular		Respiratory		Central nervous		Renal		Liver	
	BP	Inotropes	Oxygen	P/F	GCS	Sensorium	Creat	output	TB	SGOT/SGPT
Day1	N/Hypo	1/2/3/4								
Day2	N/Hypo	1/2/3/4								
Day3	N/Hypo	1/2/3/4								
Day4	N/Hypo	1/2/3/4								
Day5	N/Hypo	1/2/3/4								
Day6	N/Hypo	1/2/3/4								
Day7	N/Hypo	1/2/3/4								
Day8	N/Hypo	1/2/3/4								
Day9	N/Hypo	1/2/3/4								
Day10	N/Hypo	1/2/3/4								
Day11	N/Hypo	1/2/3/4								
Day12	N/Hypo	1/2/3/4								
Day13	N/Hypo	1/2/3/4								
Day14	N/Hypo	1/2/3/4								

Sequential organ failure assessement(SOFA) Score

Adapted from- *The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine Vincent JL et al, Intensive Care Med 1996 Jul;22(7):707-10.*

Organ system	Score				
	0	1	2	3	4
Respiratory: PaO_2/FiO_2	>400	≤400	≤300	≤200	≤100
Renal: Creatinine (mg/dl)	<1.2	1.2-1.9	2.0-3.4	3.5-4.9; urine output ≤500 ml/day	>5.0; urine output <200 ml/day
Hepatic: Bilirubin (mg/dl)	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	>12.0
Cardiovascular: Hypotension	No hypotension	MAP < 70 mmHg	Dopamine ≤ 5[a], dobutamine (any dose)	Dopamine > 5[a] or epinephrine ≤ 0.1[a] or norepinephrine ≤ 0.1[a]	Dopamine > 15[a] or epinephrine > 0.1[a] or norepinephrine > 0.1[a]
Hematologic: Platelet count (10^3/mcL)	>150	≤150	≤100	≤50	≤20
Neurologic: Glasgow coma scale score	15	13-14	10-12	6-9	<6

[a]Adrenergic agents administered for at least 1 h (doses given are in µg/kg/min). FiO_2=Fractional inspired oxygen, MAP=Mean arterial pressure, PaO_2=Arterial oxygen tension, SOFA=Sequential organ failure assessment

Annexure 4-Data sheet

Scrub IGM	Place of Ac	Antibic	Age	Sex	DOA	occupation	state	Village	med unit	dur of fe	chills	myalgia	headach	Vomiting	seizure	abdomil p	cough	breath	alt sens	bleedir	DM	HTN
1	1	2	60	1	07/02/2014	5	1	Vellore	2	10	1	2	2	1	2	2	2	1	2	2	1	1
1	1	2	54	1	01/11/2013	2	1	TV Malai	3	10	1	2	2	1	2	2	1	1	1	2	1	1
1	1	2	59	2	01/10/2014	1	1	Vellore	2	8	1	2	2	2	2	2	1	1	1	2	2	2
2	1	2	75	2	23/11/2013	1	1	Vellore	2	7	1	2	2	2	2	2	1	1	1	2	2	2
1	1	3	54	2	31/10/2013	1	1	Vellore	3	3	1	2	2	2	2	1	1	1	2	2	1	1
1	1	3	47	2	07/11/2013	1	2	Chitoor	1	7	1	2	2	2	2	2	1	1	1	2	2	2
1	1	3	38	2	06/11/2013	1	1	Vellore	4	10	1	2	2	2	2	2	1	1	1	2	2	2
1	1	3	49	2	13/12/2013	1	1	Vellore	2	10	1	2	1	1	2	2	2	1	1	2	2	2
1	1	3	24	2	03/12/2013	1	1	TV malai	4	20	1	2	2	2	2	2	1	1	1	2	2	2
2	1	3	60	2	24/11/2013	1	1	Krishgiri	3	10	1	2	2	2	2	1	2	1	1	2	2	2
1	1	3	60	1	30/10/2013	2	2	Chitoor	4	5	1	1	1	2	2	2	1	1	1	2	2	2
1	1	3	50	1	27/12/2013	2	1	Vellore	2	7	1	2	2	2	1	2	2	1	1	2	2	2
1	1	3	40	1	28/12/2013	5	1	Vellore	1	7	1	2	2	2	2	2	1	1	1	2	2	2
1	1	3	51	1	11/08/2013	5	4	KGF	3	10	1	2	2	2	2	2	2	1	1	2	2	2
1	1	3	58	1	20/10/2013	2	1	Gudiyattam	4	10	2	2	2	2	1	2	2	1	1	2	2	2
1	2	1	71	1	28/10/2013	5	2	Chittoor	4	5	1	2	2	2	2	1	1	1	2	2	2	2
1	2	1	22	1	25/11/2013	3	1	Vellore	4	10	1	1	1	2	1	2	2	1	2	2	2	2
1	2	1	50	1	27/11/2013	2	1	TV MALAI	4	5	1	1	2	2	2	2	2	1	2	2	1	2
1	2	1	55	2	05/01/2014	1	1	Vellore	2	5	1	2	2	2	2	2	1	1	2	2	2	2
1	2	1	66	2	04/01/2014	1	2	Chittoor	2	3	1	2	1	1	2	2	2	2	2	2	2	2
1	2	1	61	1	05/11/2013	5	2	Chittoor	3	10	1	2	2	2	2	2	1	1	2	2	2	1
1	2	1	47	2	10/12/2013	1	1	Gudiyattam	2	6	1	1	2	2	2	2	2	1	2	2	1	2
1	2	1	54	1	15/12/2013	5	2	Chittoor	3	15	1	2	2	2	2	1	1	1	2	2	2	2
1	2	1	58	2	04/02/2014	1	1	Walajah	2	7	1	2	2	2	2	2	1	1	2	2	2	2
2	2	1	49	2	06/12/2013	1	2	Vellore	2	10	1	1	1	2	2	2	2	1	2	2	1	1
1	2	1	54	1	22/11/2013	5	1	Vellore	2	10	1	1	1	2	2	2	2	2	2	2	1	2
1	2	1	31	1	22/11/2013	2	2	Vellore	3	15	1	1	1	1	2	2	1	1	2	2	2	2
1	2	1	42	1	10/08/2013	2	2	Chittoor	4	8	1	2	2	1	2	1	2	1	2	2	2	2
2	2	1	57	2	01/11/2014	1	1	Vellore	3	3	2	2	2	2	2	1	2	1	2	2	1	1
2	2	1	69	1	10/01/2014	5	2	Chittoor	3	3	1	2	2	1	2	2	2	1	2	2	2	2
1	2	1	33	1	01/10/2013	2	1	Vellore	4	10	1	1	1	2	2	1	2	1	2	2	2	2
1	2	1	28	1	18/10/2013	2	1	Vellore	3	11	2	1	1	2	2	2	2	1	2	2	2	2
1	2	1	70	2	10/04/2014	1	1	Vellore	3	4	1	2	2	2	2	2	1	1	2	2	2	2
1	2	1	30	1	28/03/2014	2	1	Vellore	2	7	1	1	1	2	2	2	1	2	2	2	2	2
1	2	2	75	2	28/10/2013	1	1	Katpadi	4	7	1	2	2	2	2	2	2	1	2	1	2	2
1	2	2	38	1	26/01/2014	4	1	Vellore	1	3	1	2	1	2	2	2	2	1	2	2	2	2
1	2	2	68	1	16/11/2013	5	1	Vellore	2	7	1	2	1	2	2	2	1	1	2	2	2	2
1	2	2	18	2	19/10/2013	1	1	Vellore	3	5	1	2	1	2	2	2	1	1	2	2	2	2
1	2	2	15	2	13/10/2013	4	2	Kadapa	4	7	1	2	2	2	2	2	1	1	2	2	2	2
1	2	2	22	2	28/10/2013	1	2	Chittoor	3	7	1	2	2	2	2	2	1	1	2	2	2	2
2	2	3	45	2	31/10/2013	1	1	TV MALAI	3	7	1	2	2	2	2	2	1	1	2	2	2	2
1	2	3	54	1	02/11/2013	2	1	TVMALAI	1	10	1	1	1	1	2	1	2	1	2	2	2	2
1	2	3	53	1	28/10/2013	2	1	Gudiyattam	4	10	1	1	1	1	2	2	1	1	2	2	1	2
1	2	3	28	2	30/10/2013	1	2	Chittoor	4	7	1	1	2	2	2	2	2	1	2	2	2	2
1	2	3	65	2	28/10/2013	1	1	Katpadi	4	10	1	2	2	1	2	2	2	1	2	2	1	2
1	2	3	39	1	27/11/2013	2	1	TV MALAI	4	10	1	2	2	2	2	2	1	1	2	2	2	2
1	2	3	45	1	18/10/2013	2	2	Chittoor	3	10	2	2	2	1	2	2	2	1	2	2	2	2
2	2	3	46	2	05/11/2013	1	1	Vellore	3	7	1	1	1	2	2	2	2	1	2	2	2	1
1	2	3	34	1	14/12/2013	4	1	Vellore	3	12	1	2	2	2	2	1	1	1	2	2	2	2
1	2	3	62	1	10/12/2013	2	1	Vellore	3	14	1	2	2	2	2	2	2	1	2	2	2	1
2	2	3	38	1	08/12/2013	2	2	Chittoor	1	7	1	1	2	2	2	2	2	1	2	2	2	2
1	2	3	30	2	17/11/2013	2	1	Ambur	3	7	1	2	1	2	2	1	2	1	1	2	2	2
1	2	3	35	1	19/11/2013	2	1	Katpadi	1	4	1	2	2	2	2	2	1	1	2	2	2	2
1	2	3	60	2	10/08/2013	1	3	Vellore	3	8	1	1	1	2	2	2	2	1	1	2	2	2
2	2	3	70	1	22/10/2013	4	1	Katpadi	1	30	1	2	1	1	2	2	2	1	2	2	2	2
1	2	3	55	1	10/07/2013	2	2	Chittoor	3	7	2	1	1	2	2	2	1	1	2	2	1	2
1	2	3	65	2	27/10/2013	1	1	Gudiyattam	3	10	1	2	2	2	2	2	2	1	2	2	2	2
2	2	3	45	1	23/10/2013	1	1	Vellore	1	10	2	2	2	1	2	2	2	1	2	2	2	2
1	2	3	26	2	01/08/2014	1	1	Vellore	4	7	1	2	2	2	2	2	1	1	1	2	2	2
1	2	3	47	1	19/11/2013	5	1	Vellore	1	7	1	1	2	2	2	2	1	1	2	2	2	2
1	2	3	64	2	10/01/2013	2	1	Gudiyattam	4	14	1	2	2	1	2	2	1	1	2	2	2	1
1	2	3	55	1	30/9/2013	5	1	Vellore	4	12	1	1	2	2	2	2	2	1	2	2	2	2
1	2	3	39	2	30/9/2013	1	1	Vellore	4	10	1	2	1	2	2	1	2	1	2	2	2	2
2	2	3	61	1	24/10/2013	2	2	Chittoor	1	5	1	2	2	2	2	2	2	1	2	2	1	2
1	2	1	39	2	15/08/2014	1	1	Vellore	3	8	1	1	2	1	2	2	2	2	2	2	2	2
1	2	3	60	2	28/08/2014	1	1	Vellore	3	7	1	2	2	2	2	2	2	1	2	2	2	2
1	2	1	32	2	24/08/2014	1	1	Vellore	2	8	1	1	2	2	2	2	2	1	2	2	2	2
1	1	2	75	1	02/07/2014	5	1	Vellore	1	10	1	2	2	1	2	1	2	1	1	2	2	2
1	2	2	75	1	08/08/2014	4	1	Vellore	4	4	1	2	2	2	2	2	2	1	2	2	2	2
2	2	1	32	1	05/09/2014	2	1	Vellore	4	4	1	1	1	2	2	2	2	2	2	2	2	2
1	2	1	30	1	05/09/2014	2	1	Vellore	3	10	1	2	2	2	2	2	2	1	1	2	2	2
1	2	1	51	2	21/08/2014	1	1	TV Malai	3	14	1	1	2	2	2	2	1	1	2	2	2	2
1	2	3	26	2	07/09/2014	1	1	Vellore	4	4	1	2	1	1	2	2	1	1	2	2	2	2
1	2	3	45	1	12/09/2014	2	1	Vellore	3	4	1	2	2	2	2	2	1	1	2	2	2	2
1	2	1	27	2	12/09/2014	1	1	Vellore	3	14	1	1	2	2	2	2	1	1	2	2	2	2
2	2	1	21	2	20/09/2014	3	1	Melmatta	4	5	1	1	2	1	2	2	2	2	2	2	2	2
1	2	2	30	2	23/09/2014	1	3	Kolkatta	2	1	1	1	1	2	2	2	2	1	2	2	2	2
1	2	3	17	2	23/09/2014	1	1	TVMALAI	5	3	1	1	2	2	2	2	2	1	2	2	2	2
2	1	3	40	2	25/09/2014	1	1	TVMALAI	1	7	1	1	2	2	2	2	1	1	2	2	2	2
1	1	3	68	2	21/10/2014	1	1	Vellore	1	7	1	1	2	2	2	2	2	1	1	2	2	2
2	2	2	30	1	03/10/2014	2	2	Chitoor	4	15	1	1	2	2	2	2	2	1	2	2	2	2
2	2	3	40	1	01/10/2014	2	1	Vellore	1	3	1	1	2	1	2	2	2	1	1	2	2	2
1	1	2	49	2	02/10/2014	1	1	Vellore	3	15	1	1	2	2	2	2	2	1	1	2	2	2

1	2	2	44	2	29/09/2014	1	1	Vellore	2	20	1	1	1	1	2	2	2	2	2	1	2	1
1	1	3	45	2	09/10/2014	1	2	Chitoor	1	7	1	1	2	2	2	2	2	1	1	2	2	2
1	2	2	37	2	08/10/2014	1	3	Bardhwan	2	10	1	2	2	2	2	2	2	1	2	2	2	2
1	2	3	44	2	10/10/2014	1	1	Vellore	3	5	1	1	2	2	2	2	2	1	2	2	2	2
2	2	3	80	2	03/10/2014	1	1	Vellore	4	7	1	2	2	2	2	2	2	1	1	2	2	2
2	2	2	45	2	08/10/2014	1	1	Thiruvallur	2	5	1	2	2	2	2	2	1	1	2	2	2	2
2	2	3	54	2	08/10/2014	1	1	Vellore	1	7	1	2	2	2	2	2	1	1	2	2	2	2
2	2	3	65	1	13/11/2014	5	2	Chitoor	3	3	1	2	2	2	2	2	1	1	2	2	2	2
1	2	2	23	1	07/11/2014	2	2	Chitoor	3	10	1	1	2	2	2	2	2	1	2	2	2	2
1	2	1	42	1	06/11/2014	1	1	Vellore	3	10	1	1	1	1	2	2	2	1	2	2	1	2
1	1	2	47	2	08/11/2014	1	1	TVMALAI	2	10	1	1	1	2	2	1	1	1	1	2	2	2
1	2	3	31	1	10/11/2014	5	1	TVMALAI	5	10	1	1	2	1	2	2	1	1	2	2	2	2
1	2	2	60	1	08/11/2014	2	2	Chitoor	2	10	1	1	1	2	2	2	2	1	2	2	2	2
1	2	2	55	1	08/11/2014	4	1	Vellore	2	10	1	1	2	2	2	2	1	1	2	2	2	2
2	2	1	71	1	10/11/2014	1	1	Vellore	5	5	1	1	2	2	2	2	1	1	2	2	2	2
2	2	3	49	1	08/11/2014	4	1	Vellore	1	5	1	2	2	2	2	2	1	1	2	2	2	1
1	2	1	73	2	07/11/2014	1	1	Vellore	1	8	1	1	1	2	2	2	2	2	2	2	2	2
1	1	3	72	2	01/11/2014	1	1	Vellore	1	3	1	1	2	2	2	2	1	1	2	2	2	2
2	3	3	46	2	14/11/2014	1	1	Vellore	4	2	1	1	1	1	2	2	2	1	2	2	2	2
1	1	3	62	2	23/10/2014	1	1	TVMALAI	1	3	1	2	2	1	2	2	2	1	1	2	2	2
1	2	2	20	2	22/10/2014	1	1	TVMALAI	2	15	1	2	2	1	2	2	1	1	2	2	2	2
2	2	1	60	2	03/11/2014	1	1	Vellore	5	4	1	1	1	2	2	1	2	2	2	2	1	2
1	1	2	42	1	31/10/2014	2	1	TVMALAI	4	10	1	2	2	2	2	2	1	1	1	2	2	2
1	2	3	23	2	29/10/2014	1	2	Chitoor	1	7	1	1	1	2	2	2	2	1	2	2	2	2
1	2	1	58	2	03/11/2014	1	1	TVMALAI	5	1	1	2	2	2	2	2	1	1	2	2	2	2
1	2	2	59	1	11/11/2014	2	2	Chitoor	2	10	1	1	2	2	2	2	1	1	2	2	1	1
1	2	3	58	1	01/11/2014	5	1	Vellore	4	5	1	1	1	2	2	2	2	1	1	1	1	1
2	2	1	47	2	08/11/2014	1	1	Vellore	5	7	1	1	1	2	2	2	1	1	2	2	2	2
1	2	2	57	2	08/11/2014	1	2	Chitoor	4	7	1	1	1	2	2	2	1	1	2	2	2	2
1	2	2	55	2	07/11/2014	1	1	Vellore	1	5	1	1	2	2	2	2	1	1	2	2	2	2
2	2	1	46	2	05/11/2014	1	2	Chitoor	5	10	1	1	2	2	2	2	2	1	2	2	2	2
2	1	2	49	2	28/10/2014	1	1	Vellore	2	4	1	2	2	2	2	1	2	1	1	2	2	2
2	2	3	51	2	06/11/2014	1	1	Vellore	3	6	1	1	2	2	2	2	2	1	1	2	2	2
1	1	3	47	1	04/11/2014	3	1	Vellore	2	10	1	1	2	2	2	2	2	1	1	2	2	2
2	3	1	52	2	06/11/2014	1	1	Vellore	3	10	1	1	1	2	2	2	2	1	2	2	2	2
1	1	3	50	2	29-Oct	1	1	TVMALAI	2	10	1	1	1	1	2	2	2	1	1	2	2	2
1	2	1	60	1	22/08/2014	5	2	Chitoor	1	10	1	2	2	2	2	2	1	1	2	2	2	2
1	2	1	55	1	06/11/2014	5	1	Vellore	3	15	1	1	2	2	2	1	2	1	2	2	2	2
1	1	3	70	2	07-Oct	2	1	Vellore	2	7	1	1	2	2	2	2	2	1	1	2	2	2
1	1	2	62	1	11/04/2014	3	1	Vellore	2	4	1	2	2	2	2	1	1	1	2	2	2	2
1	2	3	45	2	17/10/2014	5	2	Chitoor	3	5	1	2	2	1	2	2	2	1	2	2	2	2
2	2	1	57	2	04/11/2014	1	1	Vellore	3	5	1	1	1	2	2	2	2	1	2	2	2	1
1	1	3	48	2	01/08/2014	1	1	Vellore	1	14	1	1	2	2	2	1	2	1	1	2	2	2
1	1	3	45	2	21/08/2014	1	1	Thirupathi	3	10	1	1	2	2	2	1	1	1	1	1	2	2
1	1	3	65	1	29/09/2014	4	2	Chitoor	2	7	1	2	2	2	2	2	1	1	1	2	2	1
2	1	3	64	2	04/10/2014	1	1	Vellore	1	7	2	2	2	1	2	1	1	1	1	2	2	2
2	2	2	45	2	04/10/2014	1	1	Vellore	2	4	1	1	2	1	2	2	2	1	2	2	2	2
1	2	3	80	2	14/10/2014	1	1	Vellore	5	15	1	1	2	2	2	2	2	1	2	2	2	2
1	2	2	36	2	14/10/2014	1	1	Vellore	2	14	1	1	1	2	2	2	2	1	2	2	2	2
1	2	2	82	2	14/10/2014	1	2	Chitoor	5	7	1	2	2	2	2	2	2	2	2	2	2	2
1	1	3	52	2	14/10/2014	1	1	Vellore	5	7	1	2	2	2	2	1	2	1	1	2	2	2
1	2	2	46	2	31/12/2014	1	1	Vellore	2	7	1	1	2	1	2	2	2	1	2	2	2	2
2	2	3	60	2	27/12/2014	1	1	Vellore	4	7	1	1	1	2	2	2	1	1	1	2	2	2
2	3	1	50	1	09/12/2014	2	1	Vellore	2	11	1	1	2	2	2	2	2	2	2	2	2	2
1	2	1	28	1	09/12/2014	2	1	Vellore	5	10	1	1	1	2	2	1	1	1	2	2	2	2
1	2	2	50	2	30/11/2014	1	2	Chitoor	2	10	1	1	1	2	2	2	1	1	2	2	2	2
1	2	2	49	1	10/12/2014	2	1	Walajah	2	4	1	1	2	2	2	2	1	1	2	2	2	1
1	2	2	50	1	10/12/2014	5	2	Chitoor	2	10	1	1	2	1	2	2	2	1	2	2	2	2
2	2	3	48	2	08/12/2014	1	1	Vellore	5	10	1	1	2	2	2	2	1	1	2	2	2	2
2	2	1	59	1	06/12/2014	2	1	Vellore	4	5	1	2	2	2	2	2	1	1	2	2	1	2
1	2	3	42	2	07/12/2014	1	1	Vellore	1	10	1	1	1	1	2	2	1	1	2	2	2	2
2	1	3	45	2	07/12/2014	1	1	Vellore	3	5	1	2	2	1	2	2	2	1	1	2	2	2
1	2	1	25	1	08/12/2014	1	2	Chitoor	5	10	1	2	2	1	2	2	2	1	2	2	2	2
1	2	2	40	1	03/12/2014	5	2	Chitoor	2	15	1	1	2	2	2	2	1	1	2	2	2	2
1	2	3	27	1	06/12/2014	2	1	Vellore	3	15	1	1	1	2	2	1	1	1	2	2	2	2
1	2	1	43	1	01/12/2014	2	1	Vellore	3	14	1	1	1	2	2	2	1	1	1	2	2	2
1	2	1	30	1	28/03/2014	2	1	Vellore	2	7	1	1	2	2	2	2	1	2	2	2	2	2
1	2	1	30	2	22/11/2014	1	1	Vellore	4	10	1	1	2	2	2	2	1	1	2	2	2	2
1	1	2	45	1	22/11/2014	2	1	Vellore	4	10	1	1	2	2	2	2	2	1	1	2	2	2
1	2	3	33	1	24/11/2014	4	1	Chengam	5	7	1	1	2	2	2	2	1	1	2	2	2	2
1	2	3	45	2	24/11/2014	1	1	Vellore	5	7	1	1	1	2	2	2	2	1	2	2	2	2
2	1	3	35	2	11/12/2014	1	2	Chitoor	3	4	1	2	2	1	2	2	2	1	1	2	2	2
1	1	3	39	1	09/12/2014	5	1	Vellore	5	7	1	2	2	1	2	2	1	1	1	2	2	2
1	2	2	65	2	22/11/2014	1	1	Vellore	4	7	1	2	2	1	2	2	1	1	2	2	2	2
1	2	3	45	2	16/11/2014	1	1	Vellore	5	7	1	2	2	1	2	2	2	1	1	2	2	2
1	2	2	46	1	12/11/2014	5	1	Vellore	2	12	1	1	1	2	2	2	1	1	2	2	2	2
1	1	1	21	2	18/11/2014	1	1	Vellore	5	7	1	2	2	2	2	2	1	1	1	2	2	2
1	2	1	20	1	16/11/2014	2	1	Vellore	5	13	1	2	2	2	2	2	1	1	2	2	2	2
2	2	2	55	2	14/11/2014	1	2	Chitoor	4	4	1	2	2	2	2	2	1	1	2	2	2	2
1	1	3	25	2	16/11/2014	1	1	Vellore	3	4	1	1	2	2	2	2	1	1	1	2	2	2
2	2	3	65	2	25/11/2014	1	2	Chitoor	3	5	1	2	2	2	2	2	1	1	1	2	2	2
1	2	1	41	2	20/11/2014	1	1	Vellore	3	7	1	2	1	1	2	2	2	2	2	2	2	2
1	2	2	37	2	15/11/2014	1	1	Arrakkom	4	7	1	2	2	2	2	2	1	1	2	2	2	2
1	2	3	78	1	15/12/2014	5	2	Chitoor	5	10	1	1	2	2	2	2	2	1	2	2	2	2

2	2	1	65	1	16/12/2014	5	1	Vellore	5	5	1	1	1	1	2	2	2	1	2	2	2	2
2	2	1	42	1	18/12/2014	1	1	Gudiyattam	3	10	1	1	1	2	2	2	2	1	2	2	2	2
1	1	3	45	2	18/01/2015	1	2	Chitoor	4	10	1	1	2	1	2	2	2	1	1	2	2	2
1	1	1	30	1	07/01/2015	2	1	Vellore	1	7	1	1	2	2	2	2	1	1	2	2	2	2
1	1	2	36	1	04/01/2015	5	1	Vellore	2	15	1	2	2	2	2	2	2	1	2	1	2	2
1	1	1	45	1	08/01/2015	2	2	Chitoor	1	13	1	1	2	1	2	2	1	1	2	2	2	2
1	2	3	44	1	10/01/2015	2	1	Vellore	4	7	1	1	2	2	1	2	2	1	1	2	2	2
1	1	2	49	2	11/01/2015	1	1	Vellore	1	20	1	2	2	2	2	2	1	1	2	2	2	2
1	2	3	68	1	19/01/2015	2	1	Vellore	5	5	1	2	2	2	2	2	1	1	1	2	2	2
1	2	2	53	2	07/01/2015	1	2	Chitoor	2	10	1	1	2	2	2	2	2	1	2	2	2	2
1	1	2	31	2	19/01/2015	1	1	Vellore	5	3	1	1	2	2	2	2	1	1	1	2	2	2
1	2	2	68	2	14/01/2015	1	1	Vellore	2	25	1	1	2	2	1	2	2	1	1	2	2	2
1	1	3	24	2	12/01/2015	1	1	Vellore	5	10	1	1	2	2	2	2	2	1	1	2	2	2
1	2	2	56	2	04/01/2015	1	1	Vellore	2	10	1	1	2	2	2	1	2	1	2	2	2	2
2	1	3	67	2	05/01/2015	1	1	Vellore	5	15	1	2	2	2	2	2	1	2	2	1	1	
1	2	2	60	1	09/01/2015	2	2	Chitoor	3	7	1	2	2	1	2	1	1	1	1	2	2	2
1	1	2	43	2	08/01/2015	1	2	Chitoor	4	10	1	2	2	2	2	1	1	1	1	2	1	1
1	2	3	36	1	13/01/2015	5	2	Chitoor	5	10	1	2	2	2	2	1	1	1	2	2	2	2
1	2	3	72	2	28/12/2015	5	2	Kadapa	4	14	1	2	2	2	2	2	2	1	1	2	2	2
2	3	1	63	1	12/01/2015	2	1	TVMALAI	3	10	1	2	2	2	2	2	2	1	2	2	2	2
2	3	1	35	1	12/01/2015	5	1	Vellore	3	5	1	2	2	2	2	2	2	2	2	2	2	1
2	3	2	28	1	12/01/2015	2	1	Vellore	3	10	1	2	2	2	2	2	2	2	2	2	2	2
1	1	2	30	2	06/01/2015	1	2	Chitoor	5	9	1	2	2	2	2	2	1	1	1	2	2	2
1	2	2	29	2	13/01/2015	1	1	Vellore	2	10	1	1	2	2	2	2	2	2	2	2	2	2
1	2	3	29	1	22/01/2015	3	1	TVMALAI	4	6	1	1	2	2	2	2	2	2	2	2	2	2
1	2	2	38	1	18/01/2015	2	2	Chitoor	2	10	1	2	2	2	2	2	2	1	2	2	2	2
1	2	2	34	2	11/01/2015	1	1	Vellore	1	1	1	2	2	2	2	2	2	2	1	2	2	2
1	2	3	56	1	10/01/2015	3	2	Chitoor	4	3	1	1	1	2	2	2	2	1	1	2	2	2
1	2	2	22	2	06/01/2015	1	1	Vellore	4	2	1	1	1	2	2	2	2	1	2	2	2	2
1	2	1	65	2	09/01/2015	1	1	TVMALAI	4	14	1	1	2	2	2	2	1	1	2	2	2	2
1	3	1	20	2	05/01/2015	1	1	Vellore	5	5	1	1	1	2	2	2	2	2	2	2	2	2
1	2	2	59	1	06/01/2015	2	2	Chitoor	2	12	1	2	2	2	2	2	1	2	1	2	2	2
1	2	1	65	2	26/12/2014	1	1	Vellore	1	3	1	1	2	2	2	2	1	2	2	2	2	2
1	2	2	40	2	06/01/2015	1	1	Thiruvallur	2	5	1	2	2	2	2	2	1	1	2	2	1	2
1	2	2	65	2	27/12/2014	1	1	Vellore	4	7	1	1	1	2	2	2	2	1	2	2	2	2
1	3	1	30	1	01/01/2015	3	1	Vellore	2	4	1	1	2	2	2	2	2	2	2	2	2	2
1	3	3	62	2	03/01/2015	1	2	Chitoor	2	3	1	1	2	2	2	2	2	2	2	2	2	2
1	1	3	54	2	29/12/2014	1	1	Vellore	3	10	1	1	1	2	1	2	2	1	1	2	1	2
1	2	2	50	2	29/12/2014	1	1	TVMALAI	2	10	1	2	2	1	2	2	2	1	2	2	2	2
1	2	3	20	2	21/12/2014	1	1	Vellore	3	6	1	1	1	2	2	2	2	2	2	2	2	2
1	2	3	69	1	23/12/2014	5	1	TVMALAI	5	10	1	2	2	2	2	2	1	1	2	2	2	2
1	1	3	54	2	20/12/2014	1	1	Vellore	4	7	1	1	1	2	2	2	1	1	1	2	1	1
1	2	3	45	1	20/12/2014	5	2	Chitoor	4	14	1	1	1	2	2	2	1	1	2	2	2	2
1	2	3	19	1	15/12/2014	3	1	Vellore	4	15	1	1	2	2	2	2	1	1	2	2	2	2
1	3	1	50	2	13/12/2014	1	1	Vellore	2	5	1	1	1	2	2	2	2	2	2	2	2	2
1	2	2	65	1	06/12/2014	5	1	Vellore	4	7	1	1	2	2	2	2	2	1	1	2	2	2
1	2	1	47	1	13/12/2014	5	1	Vellore	4	15	1	1	1	2	2	2	2	1	2	2	2	2
1	2	3	55	2	05/12/2014	1	1	Vellore	3	10	1	2	2	2	2	2	1	1	2	2	1	2
1	1	2	23	2	04/12/2014	1	2	Chitoor	1	5	1	1	1	2	2	2	1	1	2	2	2	2
1	2	1	50	1	01/12/2014	5	2	Chitoor	2	5	1	1	1	2	2	2	2	1	2	2	2	2
1	1	2	54	1	28/11/2014	5	2	Chitoor	4	8	1	2	2	1	2	2	2	1	1	2	2	2
1	1	3	35	1	23/11/2014	5	1	TVMALAI	4	5	1	1	1	1	2	2	1	1	2	2	2	2
1	2	2	58	1	19/11/2014	5	1	Vellore	2	7	1	1	1	1	2	2	2	1	2	2	2	2
1	2	1	57	2	16/11/2014	1	1	Vellore	3	10	1	1	2	2	2	2	2	1	2	2	2	2
1	2	1	22	1	15/11/2014	3	1	Vellore	4	7	1	1	2	1	2	2	2	2	2	2	2	2
1	2	1	66	1	10/11/2014	5	1	Vellore	5	3	1	1	2	2	2	2	1	1	2	2	2	2

CVA	IHD	CLD	CKD	BA/COPD	Preg	smoker	alcoholic	pulse	systolic BP	diastolic BP	RR	SPO2	crepitation	Spleno	Hepatom	Rash	Eschar	Site	Hb	total cour
2	2	2	2	2	2	2	2	110	130	80	24	1	1	2	2	2	1	5	12.3	18900
2	2	2	2	2	2	2	2	152	170	90	40	2	1	2	2	2	2	0	13.4	9500
2	2	2	2	2	2	2	2	138	130	70	46	2	1	2	2	2	1	3	12.1	8300
2	2	2	2	2	2	2	2	110	70	40	36	2	1	2	2	2	1	3	9.7	11400
2	2	2	2	2	2	2	2	110	90	60	30	2	1	2	2	2	1	5	10.9	12000
2	2	2	2	2	2	2	2	110	100	70	18	2	1	2	2	2	1	2	10.5	15800
2	2	2	2	2	2	2	2	110	80	40	31	2	1	2	2	2	1	3	12	15300
2	2	2	2	2	2	2	2	110	90	60	32	2	1	2	1	2	1	5	11.6	12200
2	2	2	2	2	2	2	2	150	100	60	50	2	1	2	2	2	2	0	9.9	2400
2	2	2	2	2	2	2	2	104	130	90	38	1	1	2	2	2	1	1	11.5	16500
2	2	2	2	2	2	2	2	70	140	60	36	2	1	2	2	2	1	5	10.7	6600
2	2	1	2	2	2	2	2	138	120	80	40	2	1	2	2	2	1	2	16.6	13600
2	2	1	2	2	2	2	2	112	90	60	72	2	1	2	2	2	1	2	12.3	11900
2	2	2	2	2	2	2	2	138	90	60	46	2	2	2	2	2	1	5	13.5	10200
2	2	2	2	2	2	2	2	130	80	60	40	2	2	2	2	2	1	5	19.1	21500
2	2	2	2	2	2	2	2	84	120	70	26	1	1	2	2	2	1	2	12.2	15000
2	2	2	2	2	2	2	2	100	100	60	30	1	1	2	2	2	2	0	14.1	38000
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2	2	2	2	2	2	2	2	99	130	80	24	1	1	2	2	2	1	5	10.6	10000
2	2	2	2	2	2	2	2	99	120	80	24	1	1	2	2	2	1	3	12.6	11300
2	2	2	2	2	2	2	2	112	90	60	38	1	1	2	2	2	1	6	12.7	12100
2	2	2	2	2	2	2	2	120	100	70	40	1	1	2	2	2	1	2	11.9	13200
2	2	2	2	2	2	2	2	116	100	60	38	1	1	2	2	2	1	3	11	21600
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2	2	2	2	2	2	2	2	134	120	60	24	1	1	2	2	2	1	2	13.9	4100
2	2	2	2	2	2	2	2	110	90	60	16	2	2	2	2	2	1	2	12.6	6500
2	2	2	2	2	2	2	2	90	110	60	30	1	2	2	2	2	2	0	10.4	6600
2	2	2	2	2	2	2	2	86	94	60	30	1	1	2	2	2	2	0	17.7	11700
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2	2	2	2	2	2	2	2	70	100	60	22	2	1	2	2	2	1	1	14.6	5700
2	2	2	2	2	2	2	2	112	130	80	32	1	1	2	2	2	1	6	12.9	10000
2	2	2	2	2	2	2	1	128	100	60	36	1	1	2	2	2	2	0	14.1	9900
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2	2	2	2	2	1	2	2	108	110	80	32	1	1	2	1	2	1	3	12.2	5600
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2	2	2	2	2	1	2	2	126	90	60	30	1	1	2	2	2	1	1	10	10000
2	2	2	2	2	2	2	2	120	110	70	40	2	1	2	2	2	1	3	11.1	14500
2	2	2	2	2	2	2	2	152	90	60	42	2	1	2	2	2	1	7	13.9	8500
2	2	2	2	2	2	2	2	106	80	60	28	1	1	2	2	2	2	0	11.8	9000
2	2	2	2	2	2	2	2	120	100	70	34	1	1	2	2	2	1	3	11.8	7800
2	2	2	2	2	2	2	2	100	110	70	34	1	1	2	2	2	1	1	10.9	13400
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2	2	2	2	2	2	2	2	100	100	70	20	2	2	2	2	2	2	0	16.2	
2	2	2	2	2	2	2	2	120	130	70	24	2	2	2	2	2	2	0	14.2	11400

neutro(%)	lympho(%)	platelets	creat	urea	total bilirubin	direct bil	protein	albumin	SGOT	SGPT	ALP	Bicarb	Sofa1	dur fever d	antipyr	steroid	Total days	out at disc	statu	total hyp	total lno days
90	6	169000	1.12	49	0.6	0.4	6.8	3	79	82	121	22	3	12	2	2	6	1	1	0	0
87	10	80000	1.52	57	2.1	1.8	6.3	2.9	279	72	99	12	15	63	2	2	8	3	2	2	2
87	3	75000	1.15	72	1	0.8	5.5	2.5	54	36	130	20	12	16	2	1	9	1	1	3	3
68	28	42000	2.78	109	1.21	0.8	4.6	1.8	163	73	235	15.9	15	33	2	2	3	3	2	4	3
88	7	179000	3.76	112	0.3	0.2	6.4	2.7	13	10	61	17	6	48	2	2	8	1	1	1	0
77	19	17000	3.47	149	2.3	2.1	5.2	1.8	253	55	456	12	7	2	2	2	8	1	1	0	0
61	22	52000	2.09	97	1.3	1	5.2	1.9	51	35	286	17	11	45	2	2	8	1	1	1	1
84	13	16000	0.95	28	2	1.4	6.4	2.6	80	18	263	21.6	12	24	2	2	15	1	1	1	1
85	10	5000	0.55	69	2	1.7	5.3	2.2	228	41	171	20	13	16	2	2	16	1	1	0	0
90	6	10000	1.46	127	3.3	2.1	5.7	2	130	23	593	16	13	52	2	2	11	1	1	0	0
77	17	59000	1.06	55	0.7	0.4	5.6	2.3	69	29	432	23	8	37	2	2	11	1	1	0	0
76	15	52000	3.14	98	4.77	3.9	6	2.4	223	108	322	11	16	13	2	1	12	1	1	4	4
80	15	12000	2.07	114	2.3	2	5	2.1	225	59	211	14	18	17	2	2	7	1	1	0	0
60	32	18000	2.22	118	2.73	2.3	4.9	1.7	109	39	97	17	18	36	2	2	6	1	1	2	2
90	5	31000	2.72	121	4.4	3	6.8	2	226	93	350	10	17	6	2	2	8	1	1	4	4
72	21	145000	2.26	86	2.6	2.3	6.3	2.2	52	25	286	22	7	4	2	2	4	1	1	0	0
79	14	35000	3.07	109	2.27	2.1	5.9	2.7	307	150	202	5	10	19	2	2	5	1	1	0	0
86	10	136000	0.85	31	1.7	0.8	6.2	3.2	55	33	60	23	3	48	2	2	8	1	1	0	0
55	36	84000	0.89	32	0.7	0.5	6.9	3.2	96	53	109	19	3	4	2	2	3	1	1	0	0
69	23	8000	0.61	14	0.4	0.3	6.2	3	164	121	290	29	5	4	2	2	3	1	1	0	0
79	14	31000	2.8	132	1.25	0.8	7.1	3.1	131	90	90	14.4	10	56	2	2	9	1	1	1	0
79	15	8000	0.77	32	0.7	0.4	6.4	3	76	49	82	22	5	31	2	2	4	1	1	0	0
90	3	327000	1.47	43	0.7	0.5	6.2	2.4	27	11	207	14	2	19	2	2	8	1	1	0	0
81	13	115000	0.72	27	0.4	0.2	7	2.8	205	78	211	19.7	2	9	2	2	3	1	1	0	0
66	24	13100	0.82	26	0.66	0.2	8	4.1	53	29	138	24.3	5	55	2	2	15	1	1	0	0
75	16	101000	1.39	23	0.48	0.2	7.1	3.4	48	24	80	28.1	3	24	2	2	4	1	1	0	0
72	21	43000	0.85	20	0.4	0.2	6.1	2.5	132	100	72	21.5	4	8	2	2	2	1	1	0	0
69	24	54000	1.14	47	0.63	0.4	7.1	2.5	53	12	179	21	5	94	2	2	5	1	1	0	0
81	12	569000	0.95	26	0.3	0.2	8.1	3.4	12	10	193	23	1	4	2	2	7	1	1	0	0
82	8	111000	1.01		0.5	0.3	6.2	3.1	101	32	68	19	2	18	2	2	6	1	1	0	0
84	11	83000	1.05		7.9	6.2	6.1	3	105	60	96	20	6	8	2	2	5	1	1	0	0
78	15	104000	1.84	87	4	3.4	6.1	2.9	108	49	109	21	7	107	2	2	6	1	1	0	0
68	22	43000	1.21	62	0.5	0.2	6.1	2.6	36	6	72	19	4	17	2	2	3	1	1	0	0
68	27	91000	0.92	16	0.95	0.8	6.7	3.6	120	103	103	23	2	48	2	2	3	1	1	0	0
87	2	47000	0.79	67	0.9	0.4	5.7	1.7	100	34	99	25	4	3	2	2	6	1	1	1	0
85	10	5000	1.09	45	1.3	1	6.6	2.8	86	86	130	23	6	72	2	2	5	1	1	0	0
95	1	36000	0.93	26	1.3	0.8	6.4	3	135	39	87	28	6	81	2	2	14	1	1	0	0
80	16	83000	0.72	16	0.4	0.2	6.3	3.3	127	106	76	22	2	72	2	2	4	1	1	0	0
78	17	48000	0.84	35	0.4	0.1	6.3	2.8	76	52	107	23	5	57	2	2	3	1	1	0	0
85	12	83000	0.68	15	1.9	2	5.5	2.2	174	109	74	17	5	31	2	2	6	1	1	0	0
87	10	115000	1.02	36	0.5	0.3	5.8	2.9	85	49	162	21	2	26	2	2	9	1	1	0	0
60	31	28000	1.24	58	3.2	2.6	5.9	2.1	1845	486	236	23	9	24	2	2	6	1	1	1	0
71	25	206000	1.03	25	1.1	0.9	6.6	3.1	123	83	143	22	5	32	2	2	4	1	1	1	0
74	16	128000	0.9	20	0.7	0.5	7	3.1	124	95	258	19	3	103	2	2	4	1	1	0	0
86	11	97000	1.16	94	1.78	1.5	6.6	2	69	32	259	23	3	79	2	2	3	1	1	0	0
75	18	45000	0.97	45	1.4	1.2	6.1	2.8	82	116	92	25	4	39	2	2	3	1	1	0	0
89	4	7000	0.89	50	3.9	3.1	5.3	2.4	98	59	126	20	9	81	1	2	8	1	1	1	0
81	14	18000	0.93	24	0.36	0.1	6.4	3	104	81	99	25.5	6	31	1	2	6	1	1	0	0
81	13	6000	1.61		0.7	0.5	7.5	3	285	154	224	23	7	47	2	2	4	1	1	0	0
57	30	187000	1.78	79	1	0.8	5.6	2.4	71	39	184	17	4	27	2	2	7	1	1	0	0
55	1	12000	1.64	40	2.49	2.1	6.6	2.8	93	105	194	19.2	11	25	2	2	6	1	1	0	0
65	1	97000	0.73	25	1.73	1.3	6.5	2.6	283	149	237	24.1	6	59	2	2	5	1	1	1	0
74	21	20000	1.44		0.6	0.3	5.7	2.2	410	67	66	20	7	18	2	2	6	1	1	0	0
74	14	63000	0.92	64	3.6	2.2	5.1	2.2	189	134	261	24	6	45	2	2	4	1	1	0	0
70	23	138000	5.09	170	3.5	3.2	6	2.7	84	73	494	15	8	12	2	2	4	1	1	0	0
67	28	64000	1.69	51	1.6	0.7	5.7	2.2	77	114	91	13	9	15	2	2	5	1	1	1	0
83	9	102000	0.86	27	2.3	1.5	6.5	2.1	124	71	202	23	5	64	2	2	4	1	1	0	0
84.1	11	120000	0.74	20	0.4	0.2	6.8	2.5	37	18	124	31	1	36	2	2	3	1	1	0	0
80	14	111000	1.22	57	0.4	0.3	6.2	2.5	110	51	215	17	4	59	2	2	4	1	1	0	0
72	19	168000	1.22	30	8.29	5	5.5	2.4	114	51	162	20	7	2	2	2	3	1	1	0	0
93	4	84000	1.12	70	8.2	6.9	5.4	2.1	242	50	302	18	10	11	2	2	5	1	1	1	1
74	14	199000	1.19	42	1.3	3.1	7.2	3.1	114	45	266	25	3	54	2	2	3	1	1	0	0
83	12	116000	0.99	24	0.4	0.2	6.1	2.9	21	10	53	24	4	36	2	2	5	1	1	1	0
85	10	4000	1.2	24	1.7	0.8	7.5	3.5	83	33	82	23	6	1	2	2	2	1	1	0	0
60	36	16500	0.78	21	6.4	5.9	6.5	2.2	153	151	505	18	7	34	2	2	8	1	1	0	0
83	13	25000	5.5	155	4.8	3.5	5.4	2.3	107	39	130	13	9	26	2	2	7	1	1	0	0
74	22	67000	1.72	73	8.3	7.3	5.1	2.3	119	46	232	15	10	14	1	2	5	1	1	0	0
95	1	20000	4.52		1.87	1	4.5	1.9	109	27	155	10	19	12	1	1	10	2	2	4	4
76	13	158000	1.63	53	0.8	0.7	6.5	3.4	28	57	222	16	5	15	2	2	10	1	1	0	0
35	53	22000	1.02	30	0.49	0.3	7.1	3.9	166	71	75	24	3	54	2	2	2	1	1	0	0
57	38	150000	0.84	37	1.5	0.8	6.9	3.2	391	333	106	22	4	12	2	2	2	1	1	0	0
60	28	199000	0.69	11	0.41	0.2	6.2	3.1	46	25	101	20	0	48	2	2	4	1	1	0	0
78	15	51000	0.77	22	1.1	0.6	5.4	2.5	95	111	106		2	54	2	2	2	1	1	0	0
90	5	52000	0.83	45	0.93	0.6	6.4	3.1	92	66	164	16	5	60	1	2	4	1	1	0	0
78	18	142000	0.69	26	0.4	0.1	5.2	2.1	57	44	108	14	1	23	2	2	6	1	1	0	0
78	17	90000	0.8	17	0.46	0.3	7.8	3.6	229	139	82	24	2	47	2	2	3	1	1	0	0
71	16	118000	0.5	20	0.6	0.2	7	3.3	185	92	118	23	1	30	2	2	4	1	1	0	0
86	13	112000	0.67		2.7	2.3	5.9	2.7	118	63	481	17	6	34	2	2	5	1	1	0	0
85	11	28000	1.14	40	4.7	3.4	5.3	2.6	155	61	221	15	8	36	2	2	5	1	1	0	0
91	6	4000	0.53	51	1.1	0.8	5.7	2.8	138	63	95	21	14	95	2	2	10	1	1	2	2
73	11	7000	1.54	87	2.35	2	6.1	2.3	73	42	90	24	7	26	2	2	4	1	1	0	0
75	14	68000	0.8	14	0.76	0.6	7.3	3	169	33	83	25	2	72	2	2	3	1	1	0	0
83	13	38000	2.72	119	5.2	4.7	5.1	1.7	145	33	367	14	10	15	2	1	5	1	1	0	0

56	38	208000	0.75	13	0.3	0.2	7	3.3	139	117	240	25	1	5	2	2	8	1	1	0	0
76	18	55000	0.81	37	0.7	0.5	6	2.9	52	38	179	28	8	33	2	2	7	1	1	2	2
55	27	137000	1.2	0.6	1.6	1.4	7.1	2.5	185	54	274	23	5	12	2	2	6	1	1	0	0
62	20	313000	0.8	0.2	1.9	1.7	5.4	2.7	115	46	227	20	6	7	2	2	6	1	1	1	1
75	22	233000	2.3	223	0.4	0.2	8.1	3	28	1	68	13	2	40	2	2	7	1	1	0	0
76	19	22000	0.62	19	0.7	0.6	7	3.7	142	136	179	22	3	58	2	2	3	1	1	0	0
65	25	123000	0.95	40	0.8	0.2	6.3	3	83	93	66	23	1	27	2	2	3	1	1	0	0
84	10	126000	2.97	70	0.49	0.4	6.3	2.8	275	170	65	19	5	47	2	2	6	1	1	0	0
68	22	38000	0.98	45	2.6	2.5	4.9	1.9	198	124	151	16	5	12	2	2	6	1	1	0	0
77	12	197000	0.98	34	0.5	0.2	8.5	3.8	44	48	75	21	0	12	2	2	7	1	1	0	0
85	15	82000	1.39	89	2.52	2.6	6	2.4	237	101	362	15	15	25	2	1	18	1	1	2	2
63	29	28000	1.41	76	0.9	0.7	5.5	2.7	235	77	238	14	6	71	2	2	6	1	1	0	0
81	12	61000	2	81	4.6	4.3	6.7	2.6	75	63	450	12	4	18	2	2	10	1	1	0	0
86	14	48000	1.49	57	1.01	0.9	6.7	2.8	1.6	98	119	14.5	3	41	2	2	3	1	1	0	0
95	3	15000	1.54	138	0.5	0.2	7.1	4.4	20	12	33	15.5	5	49	2	2	3	1	1	0	0
71	22	153000	0.96	33	1.25	0.8	6.4	2.7	29	17	71	17.7	1	11	2	2	3	1	1	0	0
72	24	6000	0.85	24	0.4	0.1	5.3	3	92	62	47	18.5	5	50	2	2	4	1	1	0	0
82	14	7000	2.39	102	2	1.5	6.2	6.8	82	32	170	17.6	10	5	2	1	6	1	1	0	0
73	20	15000	0.8	26	0.69	0.5	7.5	3.6	116	101	78	19.4	4	71	2	2	0	1	1	0	0
88	7	14000	4.38	151	0.54	0.4	5.4	2.1	221	81	53	18.7	14	60	2	1	15	2	1	2	2
51	44	154000	0.97	16	0.3	0.1	6.5	2.7	97	83	78	23	3	17	2	2	7	1	1	0	0
79	15	64000	0.89	47	0.5	0.3	5.1	2.8	90	82	108	14.6	5	48	1	2	2	1	1	1	0
80	17	27000	1.13	78	4.5	3.7	5.7	2.6	295	91	298	16	16	96	1	1	4	2	2	4	4
77	18	198000	0.47	16	0.3	0.1	6.9	3.2	61	62	79	20	0	13	2	2	4	1	1	0	0
80	15	137000	0.7	45	0.32	0.3	5.7	2.6	57	46	241	17	1	72	2	2	4	1	1	0	0
69	21	269000	4.94	98	0.4	0.2	7.3	3.2	76	35	80	16	3	48	2	2	7	1	1	0	0
90	5	47000	4.73	170	4.3	3.9	5.4	2.5	112	75	140	14	10	13	2	2	14	1	1	0	0
73	22	24000	0.19	28	0.25	0.1	7.1	3.6	35	12	116	18.6	3	36	2	2	2	1	1	0	0
62	29	27000	0.69	15	0.64	0.4	6.5	3.2	48	40	118	24.4	5	7	2	2	7	1	1	0	0
70	1	153000	0.79	41	0.9	0.6	6.6	2.2	64	36	192	19	4	50	2	2	7	1	1	0	0
48	41	53000	0.72	17	0.23	0.2	6.1	2.9	72	65	97	25.7	2	23	2	2	3	1	1	0	0
82	13	55000	1.26	65	2.8	2.3	5.6	2.3	85	66	280	15	16	6	2	1	13	1	1	1	1
65	29	76000	0.65	23	9.17	8.3	6.1	2.3	24	117	228	5.3	5	25	2	2	2	1	1	0	0
64	29	20000	2.48	101	1.2	1	5.5	1.8	155	28	187	7	17	28	2	1	6	2	1	6	6
74	14	113000	0.91	31	0.23	0.1	7.2	3.1	55	66	111	18.7	1	6	2	2	0	1	1	0	0
72	14	91000	2.19	73	1.9	1.7	3.4	1.5	126	30	259	9	12	12	2	2	1	2	2	1	1
64	24	202000	0.91	31	0.56	0.5	7.4	3	63	33	162	26	0	29	2	2	3	1	1	0	0
78	15	65000	1.63	41	1.3	0.9	5.8	2.8	223	89	216	16	7	25	2	2	6	1	1	0	0
56	39	66000	2.54	143	1.03	0.5	6.5	2.7	110	18	379	25.9	15	51	2	1	7	2	2	2	2
91	6	124000	6.55	151	1.2	1	6.3	2.9	111	68	128	12	9	16	2	1	7	3	1	0	0
91	4	19000	4.73	468	7	5.6	4.4	1.8	113	49	257	12	13	11	2	2	6	1	1	0	0
78	6	59000	1.91	64	0.4	0.2	6.8	3.6	55	30	57	20	6	48	2	2	8	1	1	0	0
85	10	33000	0.77	62	3.2	3	4.7	2.7	344	64	74	18	16	14	2	2	9	1	1	2	2
70	25	29000	6.06	159	1.91	1.5	5.9	2.4	122	46	336	7	19	18	2	1	19	1	1	2	2
83	4	12000	1.65	40	3	2.5	7.2	3.1	79	49	281	15	17	71	2	2	12	1	1	3	3
88	9	42000	2.26	87	6.66	4.9	6.1	2.8	187	81	334	16	14	4	2	1	6	1	1	1	1
88	5	77000	4.2	123	2.23	2.2	6.3	2.9	127	53	322	24.5	7	3	2	2	7	1	1	0	0
72	21	268000	0.37	26	0.74	0.5	5.3	1.7	63	52	487	23	3	58	2	2	11	1	1	0	0
85	11	104000	2.04	69	1.8	1.1	6	2.4	261	124	198	21	4	30	2	2	5	1	1	0	0
72	21	141000	0.81	20	0.4	0.2	6.2	2.9	68	20	127	25	1	26	2	2	6	1	1	0	0
86	10	24000	3.42	119	2.8	1.7	5.5	2.4	83	24	254	8	16	39	2	1	10	1	1	1	1
79	21	86000	0.92	30	1.8	1.1	6.5	3.2	114	168	212	19	5	58	2	2	4	1	1	0	0
70	1	78000	3.17	93	3.7	2.4	6.2	3.3	64	43	148	16	8	47	2	2	5	1	1	0	0
76	20	62000	1.04		0.44	0.2	7.8	3.5	5	133	223	25.7	2	45	2	2	0	1	1	0	0
79	17	92000	2.51	79	0.8	0.5	6	2.9	164	80	89	16	4	12	1	2	4	1	1	0	0
76	19	26000	1.34	52	0.7	0.1	0.4	2.5	92	74	112	19.6	4	14	1	2	3	1	1	0	0
87	10	45000	1.73	63	0.97	0.8	6.8	2.6	413	94	68	18.5	6	51	1	2	5	1	1	0	0
42	51	40000	2.59	113	8.64	8.2	7	2.7	297	122	330	18.7	10	50	1	2	9	1	1	0	0
92	5	84000	0.61	27	5.31	4.9	5.8	1.6	321	60	385	12	4	30	1	2	8	1	1	0	0
60	30	153000	1.46		0.55	0.3	7.3	2.8	89	152	68	22.3	1	35	2	2	3	1	1	0	0
86	8	44000	0.91	43	0.8	0.6	6.9	2.6	153	72	78	15	3	13	2	2	2	1	1	0	0
59	34	16000	0.65	58	3.4	3.1	7.5	2.6	393	69	129	15	16	28	2	1	3	2	1	3	3
63	20	82000	0.68	12	1.2	0.3	7	3.6	65	19	84	23	3	47	2	2	3	1	1	0	0
74	24	9000	0.7	43	1.16	0.7	8.3	2.9	101	43	142	25.8	4	47	2	2	6	1	1	0	0
71	21	122000	1	24	0.77	0.4	7.6	4.3	95	71	93	22	2	55	2	2	4	1	1	0	0
82	15	134000	0.93	56	0.35	0.1	7.6	2.8	82	52	63	20	3	24	2	2	12	1	1	0	0
68	27	91000	0.92	16	0.95	0.8	6.7	3.6	120	103	103	23	2	48	2	2	3	1	1	0	0
84	10	17000	0.72	2	0.62	0.5	6.4	2.8	127	35	100	23.2	4	47	2	2	4	1	1	0	0
62	34	26000	3.53	138	12.6	11.3	6	2.7	245	126	439	16	19	144	2	1	9	2	1	3	3
69	27	117000	1.13	24	1.18	0.9	5.9	2.5	273	155	111	20	1	40	2	2	5	1	1	0	0
75	17	52000	0.68	35	0.48	0.2	6.7	2.9	190	55	78	22	4	26	2	2	5	1	1	0	0
68	28	88000	0.74	67	4.39	4	5.2	2.1	376	160	224	17	11	11	2	2	6	1	1	0	0
85	14	36000	3.39	137	7.3	6.2	5.6	2.3	167	53	125	12	19	62	2	1	11	1	1	2	2
67	30	133000	2.85	116	0.6	0.4	7.6	2.4	39	11	263	7	7	30	2	2	4	1	1	0	0
66	2	55000	1.73	128	0.7	0.3	4.9	1.9	63	24	111	14	9	39	2	2	8	1	1	2	2
74	16	118000	1.08	36	2.87	2.7	6.7	3	69	51	209	22	3	42	2	2	7	1	1	0	0
65	27	53000	0.84	48	0.7	0.1	5.6	2.2	137	16	178	20	5	17	2	2	6	1	1	0	0
72	19	15000	1.03	39	0.9	0.5	6.6	2.5	150	68	126	22	4	34	2	2	6	1	1	0	0
92	5	63000	1.6	69	2.8	2.1	4.7	2.6	94	68	198	18	5	69	2	2	8	1	1	0	0
76	19	106000	0.65	27	0.6	0.2	5.8	2.6	87	46	159	17	13	7	2	1	10	1	1	2	2
90	6	45000	1.81	113	4.1	3.8	5.6	1.7	86	27	235	18	6	43	2	2	5	1	1	0	0
38	53	153000	0.64	18	0.3	0.1	8	3.7	58	43	171	23	0	44	2	2	4	1	1	0	0
75	21	158000	0.56	15	0.6	0.5	5.2	2.2	53	15	253	18	3	11	2	2	3	1	1	0	0
86	6	140000	2.95	106	1.1	0.4	7.1	3.5	200	166	85	21.6	3	18	2	2	5	1	1	0	0

87	4	67000	1.21	61	5.64	4.6	5.6	2.3	153	40	103	17	6	12	2	2	2	1	1	0	0
59	28	6000	0.85	35	0.9	0.2	8	3.2	63	65	94	28	4	36	2	2	2	1	1	0	0
90	7	180000	2.19	70	5	4.9	6.7	2.8	115	41	45	10	16	50	2	1	6	2	1	6	6
72	24	15000	0.82	30	0.76	0.7			63	148		23	4	18	2	2	5	1	1	0	0
76	17	80000	1.51	48	7.4	6.7	6.3	3	153	134	219	20	6	96	2	2	7	1	1	0	0
82	16	43000	1.2	35					136	332		17	4	21	2	2	3	3	1	1	0
81	16	163000	1.92	100	0.6	0.4	8.4	2.9	96	45	123	17	9	41	2	2	11	1	1	0	0
84	14	126000	1.15		1	0.6	6.5	2.5	95	26	91	17	2	34	2	2	4	1	1	0	0
60	31	10000	3.02	15	3.9	2.7	6.5	2.8	170	50	314	9.9	20	31	2	2	11	1	1	1	1
82	14	6000	0.79	52	0.77	0.7	5.2	2.1	51	41	322	19	4	23	2	2	13	1	1	0	0
92	6	29000	0.5	26	0.59	0.4	4.5	1.8	135	41	205	14	11	41	2	1	11	1	1	0	0
86	11	57000	1.7	126	5.5	4.9	6.4	2.2	188	39	733	13.6	5	25	2	2	10	1	1	0	0
50	40	68000	0.9	63	2.1	1.8	5.3	2.7	208	56	198	15	15	35	2	1	9	1	1	3	3
58	40	60000	1.06	87	1.7	15	6.5	2.6	62	18	176	20	3	21	2	2	6	1	1	0	0
82	13	73000	1.77	64	0.57	0.3	6.8	2.9	350	138	102	13.5	6	22	2	1	5	1	1	1	1
86	10	7000	2.47	138	5.8	4.2	5.5	2.3	133	70	120	11.3	20	6	2	1	1	2	1	1	1
51	39	5000	0.71	38	2.2	1.3	6.9	2.9	108	88	284	19.6	16	1	2	1	17	1	1	3	3
59	38	26000	0.79	42	3.7	3.1	7.4	3.2	783	245	261	19	10	24	2	2	4	1	1	0	0
85	10	169000	0.58	23	0.8	0.6	5.8	2.4	492	121	603	25	13	60	2	2	11	2	1	11	11
76	15	88000	1.23		0.7	0.4	7.1	2.9	95	63	50		3	28	2	2	0	1	1	0	0
74	16	369000	0.76		2	0.4	8.6	4.8	15	38	102	21.3	2	30	2	2	0	1	1	0	0
70	24	14000	0.67	9	0.4	0.1	7.3	3.5	47	54	119	20	4	40	2	2	0	1	1	0	0
81	17	118000	0.76	43	1.1	0.7	4.5	2.2	228	130	298	17	14	51	2	2	10	1	1	1	1
42	53	129000	0.4	120	3.15	3.3	6.5	2	112	41	572	28	3	36	2	2	7	1	1	0	0
73	21	98000	1.12	33	1.5	1	6.2	3.1	223	88	96	23	3	36	2	2	4	1	1	0	0
71	10	72000	1.24		8.33	7.1	6.7	2.2	210	85	1023	19.4	8	48	2	2	4	1	1	0	0
86	12	56000	0.49	27	0.8	0.2	8.2	3.9	48	42	51	19	4	30	2	2	9	1	1	0	0
72	21	175000	2.68	124	6.12	5.5	5.4	2.4	96	27	241	14.4	9	21	2	2	4	3	1	0	0
91	2	18000	2.6	129	0.86	0.8	4.3	1.5	149	35	389		6	24	2	2	10	1	1	0	0
74	20	81000	0.97	56	3.4	1.9	6.1	2.2	197	76	206	22	6	60	2	2	4	1	1	0	0
68	18	69000	0.91		0.33	0.2	8.2	3.4	116	76	84	26	3	24	2	2	0	1	1	0	0
63	30	348000	0.76	40	1.6	0.8	6.7	3.4	282	443	259	20	2	24	2	2	4	1	1	0	0
37	4	48000	1.49	66	0.58	0.1	6.2	3	168	58	41	23	4	49	2	2	4	1	1	0	0
93	4	59000	0.92	30	0.94	0.8	6.4	2.9	106	53	168	26	4	12	2	2	7	1	1	0	0
90	1	26000	0.76	49	0.9	0.2	5.9	2.5	57	22	116	19.8	3	72	2	2	4	1	1	0	0
78	13	124000	0.89	15	0.9	0.5	6.4	3.6	101	60	94	23	1	12	2	2	0	1	1	0	0
67	25	162000	0.63	27	0.8	0.5	6.3	2.9	71	58	220	22	2	20	2	2	0	1	1	0	0
92	7	79000	4.44	63	4.8	4	7	2.6	62	27	402	16	11	40	2	1	10	1	1	0	0
66	24	146000	0.73	26	0.48	0.3	6.3	2.9	58	29	107	24	2	120	2	2	7	1	1	0	0
68	28	135000	0.61	22	0.9	0.8	7.4	2.6	91	61	234	23	3	47	2	2	4	1	1	0	0
63	30	34000	1.55	71	6	5.3	6.1	2.2	98	53	240	25	8	34	2	2	4	1	1	0	0
60	26	143000	4.65	121	0.4	0.2	6.6	2.8	36	27	136	10	10	70	2	1	15	1	1	0	0
64	35	34000	1.31	45	0.4	1.4	5.7	2.6	158	93	135	16	6	96	2	2	4	1	1	0	0
62	35	22000	1.29	85	0.9	0.4	5.9	2.4	130	30	154	20	6	72	2	2	4	1	1	0	0
55	37	232000	0.45		0.38	0.2	6.5	2.7	276	278	125	19.6	1	28	2	2	0	1	1	0	0
73	24	136000	1.55	131	0.3	0.1	6.3	2.6	118	41	59	12	7	48	2	2	18	1	1	1	1
90	6	125000	5.74	159	1.25	1	8.8	3.1	62	17	393	16	5	24	2	2	6	1	1	0	0
72	23	130000	0.61	15	0.5	0.4	6.2	3	69	33	250	24	3	56	2	2	8	1	1	0	0
46	51	74000	0.72	29	0.4	0.3	5.1	1.8	49	22	175	16	4	70	2	2	5	1	1	0	0
68	23	92000	0.98	26	0.84	0.4	7	3.8	68	128	113	25	2	24	2	2	3	1	1	0	0
70	20	51000	2.08	119	11.2	10.1	6.3	2.4	459	151	333	16.8	10	48	2	1	8	1	1	2	2
84	11	1000	0.83	29	1.8	1	6	2.7	204	78	50	22	6	60	2	2	9	1	1	0	0
72	18	5000	1.12	36	1.1	0.5	6.8	3.4	102	94	155	22	4	40	2	2	5	1	1	0	0
86	10	101000	0.77	36	0.7	0.5	6	2.6	99	59	93	22.7	1	22	2	2	5	1	1	0	0
		16000	0.91		1.1	0.3	7.2	4.2	58	56	156		4	20	2	2	4	1	1	0	0
61	34	218000	0.72	32	1.18	1	7.1	3	61	43	124	20.8	1	10	2	2	4	1	1	0	0

total Oxy d	NIV days	IV days	total gc	total sen	1Total crei	2Total cre	total out	1Total tb>2 days
4	4	0	0	0	0	0	0	0
8	0	8	8	8	1	7	0	1
8	0	7	7	7	0	0	0	0
4	0	0	1	1	0	2	0	0
2	2	0	0	0	1	2	0	0
5	5	0	4	4	0	3	0	6
6	5	0	5	6	2	1	1	0
11	1	10	10	10	0	0	0	2
8	0	8	6	6	0	0	0	6
9	0	9	9	9	1	0	0	4
6	0	5	5	5	0	0	0	0
7	0	7	5	5	0	10	0	6
4	0	4	4	4	1	1	0	5
3	0	3	3	3	0	1	0	2
7	0	7	5	5	1	5	0	4
1	0	0	0	0	1	1	0	1
1	0	0	0	0	1	1	0	1
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
2	0	0	0	0	2	3	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
4	2	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	5
6	6	0	0	0	1	0	0	6
1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
3	2	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	3
2	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	1
1	0	0	0	0	0	0	0	0
1	0	0	0	0	1	0	0	0
2	2	0	0	0	2	0	0	0
4	3	0	0	0	1	0	0	3
3	0	0	2	2	0	0	0	0
5	0	0	0	0	0	0	0	0
1	0	0	1	1	0	0	0	1
0	0	0	0	0	0	4	1	5
1	0	0	0	0	1	0	0	0
1	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0
1	0	0	1	1	0	0	0	0
1	0	0	0	0	0	0	0	3
3	0	0	0	0	0	0	0	4
1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	4
0	0	0	0	0	1	6	6	6
0	0	0	0	0	2	0	0	4
10	0	10	10	10	8	8	8	5
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	1	1	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
3	2	0	0	0	0	0	0	3
4	3	0	0	0	0	0	0	4
9	0	9	8	8	0	0	0	0
0	0	0	0	0	0	0	0	3
0	0	0	1	1	0	0	0	0
4	0	0	2	2	4	4	0	4

0	0	0	0	0	0	0	0	0
5	0	4	4	4	0	0	0	0
3	2	0	0	0	0	0	0	0
2	0	0	0	0	2	1	0	0
0	0	0	2	2	0	2	2	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
3	0	0	0	0	3	2	0	0
0	0	0	0	0	0	0	0	6
0	0	0	0	0	0	0	0	0
7	0	7	6	6	0	0	0	4
6	2	0	0	0	0	0	0	0
0	0	0	0	0	2	2	0	10
0	0	0	0	0	1	0	0	0
0	0	0	0	0	3	2	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
4	2	0	0	0	2	2	2	2
0	0	0	0	0	0	0	0	0
15	0	15	15	15	10	8	8	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
4	0	4	4	4	0	0	0	4
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	3	2	0	0
1	0	0	1	1	3	3	0	6
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
7	2	5	5	5	0	0	0	5
0	0	0	0	0	0	0	0	2
6	0	6	6	6	6	6	5	0
0	0	0	0	0	0	0	0	0
1	0	1	1	1	1	1	1	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
7	0	7	5	5	5	3	3	0
0	0	0	0	0	7	7	7	0
2	2	0	0	0	5	4	2	4
1	0	0	0	0	3	0	0	0
6	0	6	6	6	0	0	0	3
6	0	5	6	6	10	8	2	3
8	0	8	8	8	4	0	0	7
4	0	4	4	4	4	1	2	6
0	0	0	0	0	2	2	0	3
6	2	0	0	0	0	0	0	0
0	0	0	0	0	4	3	0	0
0	0	0	0	0	0	0	0	0
7	0	7	7	7	3	3	0	3
0	0	0	0	0	0	0	0	0
3	0	0	1	1	5	3	0	5
0	0	0	0	0	0	0	0	0
0	0	0	0	0	3	3	0	0
0	0	0	0	0	0	0	0	0
2	0	0	0	0	2	0	0	0
2	0	0	0	0	3	2	2	5
0	0	0	0	0	0	0	0	8
0	0	0	0	0	3	0	0	0
0	0	0	0	0	0	0	0	0
3	0	3	3	3	0	0	0	3
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
2	0	0	1	1	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
4	0	4	3	3	9	9	3	9
0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	3	3	3	0	0	0	6
6	0	6	6	6	3	2	0	7
2	2	0	0	0	2	1	0	0
6	0	0	1	1	8	5	0	0
0	0	0	0	0	0	0	0	7
4	0	4	4	4	0	0	0	0
0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	3
6	0	6	6	6	0	0	0	0
0	0	0	0	0	3	0	0	5
0	0	0	0	0	0	0	0	0
2	2	0	0	0	0	0	0	0
0	0	0	0	0	4	3	0	0